Using flow cytometry to investigate microbiome biology

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ESCCA 2022 Belfast
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APC Microbiome Ireland Team

300 People…our most important asset

Multiple basic research and clinical disciplines applied to same problem

Microbiology, immunology, pharmacology, neuroscience, food science, nutrition, biochemistry, medical microbiology, pharmacy, physiology
Gastroenterology, psychiatry, cardiovascular health, rheumatology, radiology, oncology, pathology, gerontology, neonatology, metabolic health

Bench
to
Bedside

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APC Mission

To bring talented researchers, clinicians and industry together to develop microbiome based knowledge, solutions and products to tackle societal challenges and support economic growth.

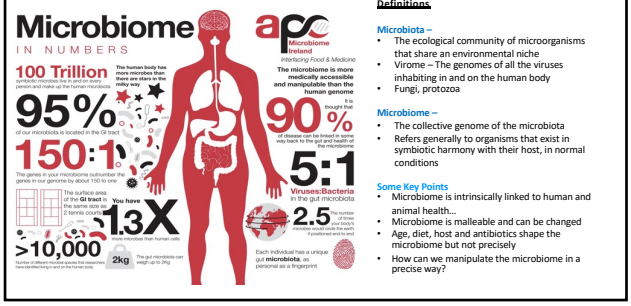
3

PIs by APC Research Themes

THEME 1	THEME 2	THEME 3	THEME 4
MICROBES TO MOLECULES	DIET & MICROBES	BRAIN-GUT-MICROBIOTA AXIS	HOST MICROBE DIALOGUE
PI Co-Leads Colin Hill	PI Co-Leads Catherine Stanton	PI Lead: John Cryan	PI Lead Subrata Ghosh
Douwe van Sinderen	Paul O'Toole	Co-PI Gerard Clarke	Co-PIs Fergus Shanahan
Co-PIs Paul Ross	Co-PIs Paul Cotter		Cormac Gahan
Ruth Massey	Ines Thiele		Noel Caplice
Andrey Shkoporov	Eike Arendt		Liam O'Mahony
Jennifer Mahony	Orla O'Sullivan		Ken Nally
	Jens Walter		Marcus Claesson

4

We live in a microbial world & are all different



Definitions

Microbiota –
- The ecological community of microorganisms that share an environmental niche
- Virome – The genomes of all the viruses inhabiting in and on the human body
- Fungi, protozoa

Microbiome –
- The collective genome of the microbiota
- Refers generally to organisms that exist in symbiotic harmony with their host, in normal conditions

Some Key Points
- Microbiome is intrinsically linked to human and animal health..
- Microbiome is malleable and can be changed
- Age, diet, host and antibiotics shape the microbiome but not precisely
- How can we manipulate the microbiome in a precise way?

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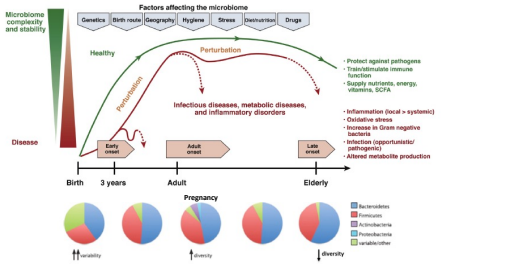
Factors Affecting the Microbiome Across the Human Life Cycle



Adapted from Kostic et al., 2013 Gene Dev & Kostic et al., 2014 Gastroenterology

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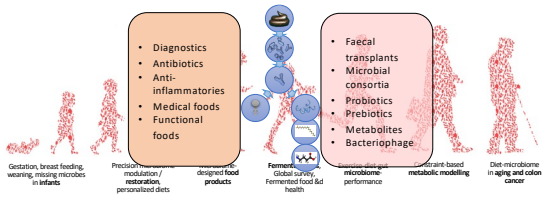
Mining the Microbiome for interventions across the life cycle



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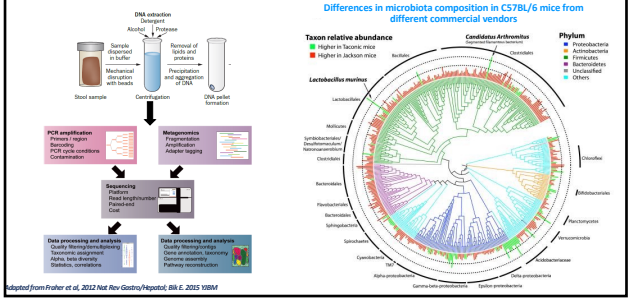
Analysing the Microbiota Composition



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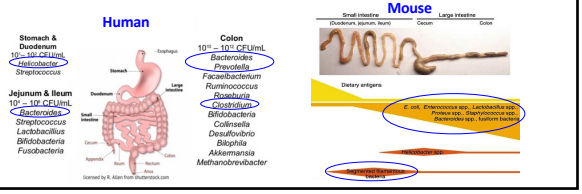
Microbiota of the Gastrointestinal Tract Differences between Mouse and Human

- Largest numbers of microbes and greatest variety of species
- Approximately 800-1000 bacterial species in the human gut
- Four dominant bacterial phyla
  - Firmicutes (64%), Bacteroidetes (23%), Proteobacteria (8%) and Actinobacteria (3%)
  - Firmicutes – Gram positive bacteria; Clostridia
  - Bacteroidetes – Gram negative bacteria; Bacteroides, Prevotella



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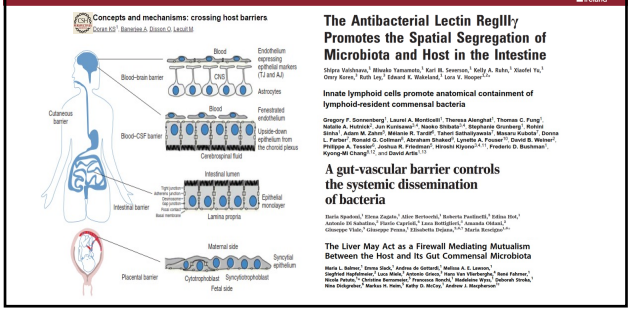
Host Barriers Prevent Systemic Dissemination of Gut Commensal Bacteria



The Antibacterial Lectin RegIIIγ Promotes the Spatial Segregation of Microbiota and Host in the Intestine

Shipra Vaishnava,[1] Mineko Yamamoto,[1] Kari M. Severson,[1] Kelly A. Ruhn,[1] Xiaofei Yu,[1] Omry Koren,[2] Ruth Ley,[2] Edward K. Wakeland,[1] Lora V. Hooper[1,*]

Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria

Gregory F. Sonnenberg[1], Laurel A. Monticelli[1], Theresa Alenghat[1], Thomas C. Fung[1], Natalie A. Hutnick[1], Jun Kunisawa[2,3], Naoko Shibata[2,3], Stephanie Grunberg[1], Rohini Sinha[1], Adam M. Zahm[1], Mélanie R. Tardif[4], Taheri Sathaliyawala[1], Masaru Kubota[1], Donna L. Farber[1], Ronald G. Collman[1], Abraham Shaked[4], Lynette A. Fouser[5], David B. Weiner[1], Philippe A. Tessier[6], Joshua R. Friedman[6], Hiroshi Kiyono[2,3,7], Frederic D. Bushman[1], Kyong-Mi Chang[1,*], and David Artis[1,8,*]

A gut-vascular barrier controls the systemic dissemination of bacteria

Boris Spadoni,[1] Elena Zagato,[1] Alice Bertocchi,[1] Roberta Paolinelli,[2] Edlira Hot,[1] Antonio Di Sabatino,[3] Flavio Caprioli,[4] Luca Bottiglieri,[1] Amanda Oldani,[1] Giuseppe Viale,[5] Giuseppe Penna,[1] Elisabetta Dejana,[1,6,7] Maria Rescigno[1,8,*]

The Liver May Act as a Firewall Mediating Mutualism Between the Host and its Gut Commensal Microbiota

Maria L. Balmer,[1] Emma Slack,[1] Andrea de Gottardi,[2] Melissa A. E. Lawson,[1] Siegfried Hapfelmeier,[1] Luca Miele,[3] Antonio Grieco,[3] Hans Van Vlierberghe,[4] René Fahrner,[5] Nicola Patuto,[6] Christina Bernsmeier,[2] Francesco Rocchi,[7] Madeleine Wyss,[1] Deborah Stroka,[2] Nina Dickgreber,[8] Markus H. Heim,[9] Kathy D. McCoy,[1] Andrew J. Macpherson[1,9]

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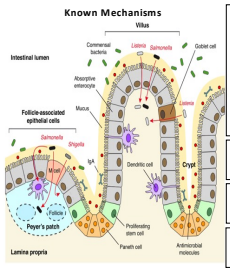
Crossing Host Barriers: Systemic Dissemination by Pathogens



Salmonella typhymurium

Direct uptake through peyers patches and inter-epithelial DCs and dissemination to MLN and Spleen
(Rescigno M et al, Nat Immunol. 2001 Apr;2(4):361-7)

Transcytosis (apical to basolateral translocation in epithelial cells)
(Andreas JM et al, Cell Host Microbe. 2012 Jan 19;11(1):19-32)

Self destructive co-operation
(Martin A et al, Nature 454, 987-990 (21 August 2008))

Destruction of M cells
(Jone BD et al, J Exp Med.1994 Jul 1;180(1):15-23)

Listeria monocytogenes

Transcytosis (apical to basolateral translocation in epithelial cells)
(Andreas JM et al, Cell Host Microbe. 2012 Jan 19;11(1):19-32)

Enteropathogenic *E. coli*

Destabilize tight junctions and adherens junctions
(Yarbrough ML et al Science. 2009 Jan 9;323(5911):269-72)

Shigella flexineri

Destruction of M cells
(Jone BD et al, J Exp Med.1994 Jul 1;180(1):15-23)

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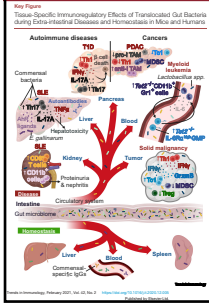
Crossing Host Barriers: Systemic Dissemination by Commensal Bacteria



Immunity

Lymphoid-Tissue-Resident Commensal Bacteria Promote Members of the IL-10 Cytokine Family to Establish Mutualism

Programmable probiotics for detection of cancer in urine

Translocation and dissemination of commensal bacteria in post-stroke infection

Translocation of a gut pathobiont drives autoimmunity in mice and humans

SCIENTIFIC REPORTS

Brain microbiota disruption within inflammatory demyelinating lesions in multiple sclerosis

The human tumor microbiome is composed of tumor type–specific intracellular bacteria

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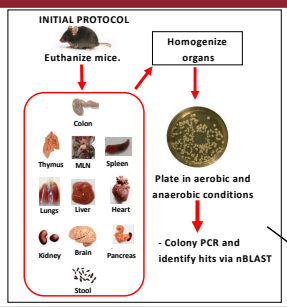
Method to Identify and Characterise Commensal Bacteria Residing in Mouse Mucosal and Systemic Tissue



Bacillus cereus- Environmental contaminant

Micrococcus luteus- Environmental/Mammalian skin flora

Staphylococcus capitis- Mammalian skin flora

Organ	Bacteria (Blast hit)
Liver	*Bacillus cereus*
MLN	*Micrococcus luteus*
WAT	*Micrococcus luteus*
Kidney	*Micrococcus luteus*
Pancreas	*Staphylococcus capitis*
Caecum	*Micrococcus sp.*
Thymus	*Staphylococcus capitis*
Lungs	*Staphylococcus capitis*
Pancreas	*Bacillus cereus*
Pancreas	*Bacillus species*

MLN- Mesenteric lymph Nodes, WAT- White adipose tissue

Summary:
Workflow- Not sufficient to prevent contamination

All Steps were performed in Laminar Flow Hood Using Aseptic Tools

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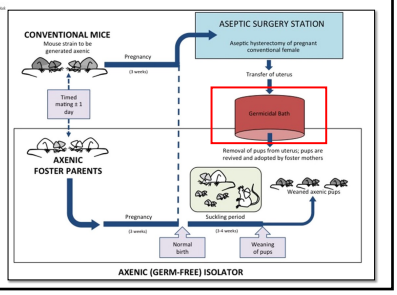
Modification of Workflow- Incorporation of Germicidal Bath from Germ Free Mice Generation

A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome



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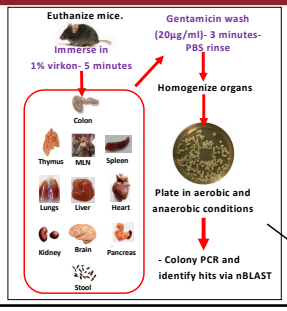
An Aseptic Culture-based Approach to Identify Bacteria in Systemic Tissues of Mice



Germ free (n=9)

3 colonies of *Bacillus sp.* (possible spore-resistant air contaminants) from 108 tissues total

Summary:
Workflow- Sufficient to prevent contamination

All Steps were performed in Laminar Flow Hood Using Aseptic Tools

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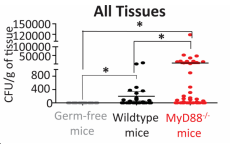
Gut bacteria naturally translocate to systemic tissues of WT and MyD88$^{-/-}$ Mice

	WT C57BL/6 (SPF) (n = 6), males: 21-23 weeks	MyD88$^{-/-}$ (n = 6), males: 21-23 weeks
HEART	*L. johnsonii, B.pseudolongum, F. rodentium*	*L. johnsonii*
THYMUS	*B.pseudolongum*	*L. johnsonii*
LIVER	*L. johnsonii, B.pseudolongum*	*S. danielae, L. johnsonii, L. murinus*
SPLEEN	*L. johnsonii, B.pseudolongum*	*L. reuteri, L. johnsonii*
PANCREAS	*B.pseudolongum*	*L. johnsonii*
KIDNEY	*B.pseudolongum*	*L. johnsonii*
WAT	-	Uncharacterized bacteria
MLN	-	*L. reuteri, B. pseudolongum*



***Lactobacillus johnsonii*: The most recurring hit in systemic tissues**

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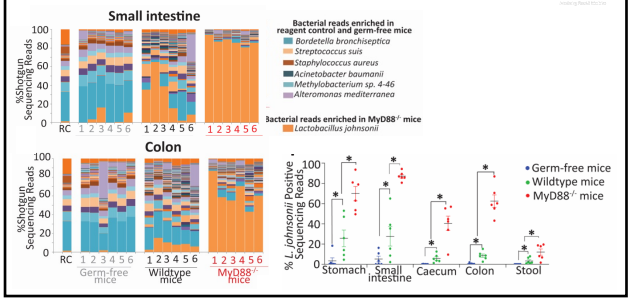
L. johnnsonii colonize intestinal tissues of WT and MyD88$^{-/-}$ mice, with higher colonization of MyD88$^{-/-}$ mice



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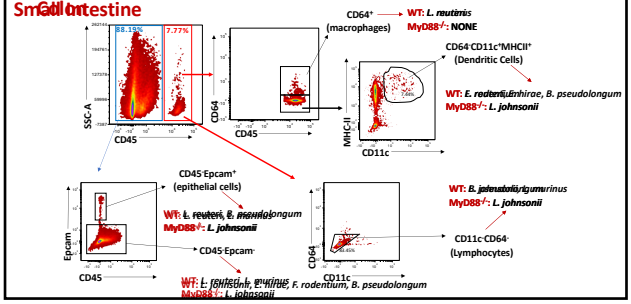
Identification of Bacteria in Sorted Cells of Intestinal Tissues



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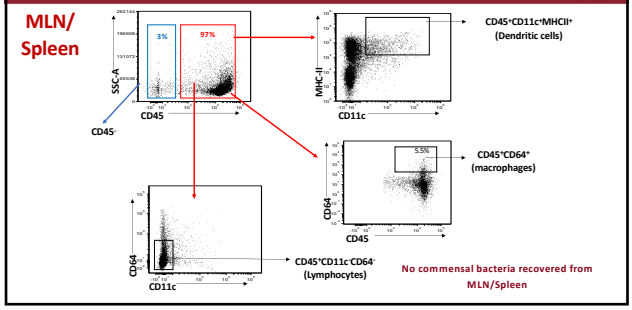
Identification of bacteria in sorted cells of systemic tissues



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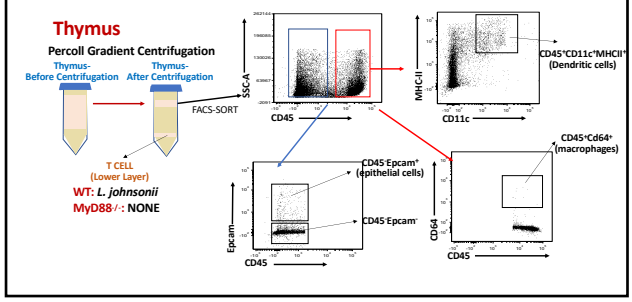
Identification of bacteria in sorted cells of systemic tissues



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Identification of bacteria in sorted cells of intestinal tissues

Cell Type	Mice	WT mice (Bacteria Recovered)	MyD88$^{-/-}$ mice (Bacteria Recovered)
Epithelial	Small Intestine	*L. reuteri, B. pseudolongum*	*L. johnsonii*
	Colon	*L. murinus, L. reuteri*	*L. johnsonii*
Stromal	Small Intestine	*L. reuteri, L. murinus, C. acnes*	*L. johnsonii*
	Colon	*L. johnsonii, B. pseudolongum, E. hirae, F. rodentium*	*L. johnsonii*
Macrophage	Small Intestine	*L. reuteri*	-
	Colon	*Uncultured bacterium, L. murinus*	-
Dendritic Cell	Small Intestine	*L. reuteri, E. hirae, B. pseudolongum*	*L. johnsonii*
	Colon	*F. rodentium*	*L. johnsonii*
Lymphocytes	Small Intestine	*L. johnsonii, L. murinus, C. acnes*	*L. johnsonii*
	Colon	*B. pseudolongum*	*L. johnsonii*

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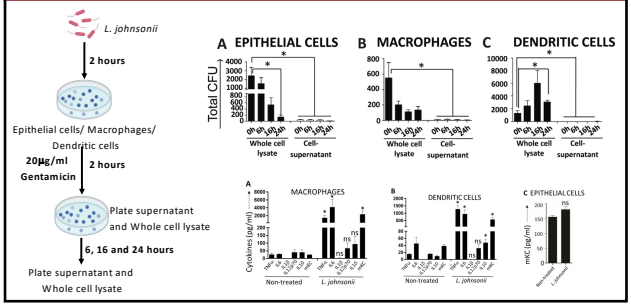
L. johnsonii persist in primary DCs but not macrophages or epithelial cells



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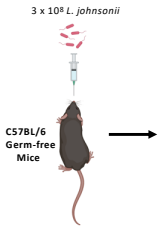
Orally gavaged *L. johnsonii* translocate to systemic tissues of germ-free mice



Organ		*L. johnsonii* positive tissues (n=3)	CFU/g
Intestinal tissues	Stomach	3/3	213333, 200000, 586666
	Small Intestine	3/3	228571, 180000, 97142
	Caecum	3/3	533333, 360000, 400000
	Colon	3/3	260000, 160000, 170000
	Stool	3/3	880000, 1440000, 1920000
Systemic tissues	Liver	1/3	0, 143, 0
	Pancreas	1/3	0, 5500, 0
	Kidney	1/3	950, 0, 0
	MLN*	2/3	500, 3100, 0
	WAT**	2/3	0, 7200, 100

*MLN- Mesenteric lymph Nodes, **WAT- White adipose tissue

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Summary

- Systemic dissemination of live gut-resident bacteria occurs to varying degrees between co-housed mice.
- *L. johnsonii* is the most recurring bacteria recoverable from systemic tissues of WT and MyD88$^{-/-}$ mice.
- Systemic dissemination of *L. johnsonii* in MyD88$^{-/-}$ mice is higher compared to WT mice, due to their higher intestinal abundance in these mice.
- *L. johnsonii* strains are recoverable from multiple cell types of intestinal tissues and persist intracellularly in dendritic cells but not macrophages or epithelial cells.

› Gut Microbes. Jan-Dec 2022;14(1):2007743. doi: 10.1080/19490976.2021.2007743.

Identification of Gut Bacteria such as Lactobacillus johnsonii that Disseminate to Systemic Tissues of Wild Type and MyD88−/− Mice

Sreeram Udayan [1,2,3], Panagiota Stamou [1], Fiona Crispie [1,4], Ana Hickey [1,2], Alexandria N Floyd [3], Chyi-Song Hsieh [5], Paul D Cotter [1,4], Orla O'Sullivan [1,4], Silvia Melgar [3], Paul W O'Toole [1,6], Rodney D Newberry [3], Valerio Rossini [1], Ken Nally [1,2]

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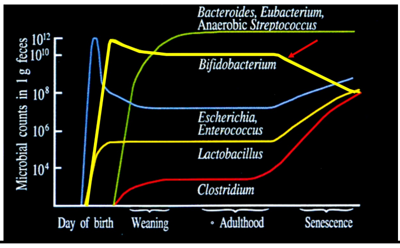
Bifidobacterial abundance in the human gut

- High GC-content, anaerobic bacteria
- *Bifidobacterium* is a deep-branching lineage of the phylum *Actinobacteria* (now *called: Actinomycetota*)
- The genus currently consists of approximately 100 taxa (species/subspecies)

Prof Tomotari Mitsuoka ,
1973 (classical cultivation)



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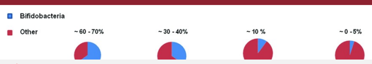
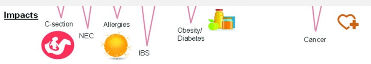
Bifidobacteria decline as its host ages



- ➢Their absence is linked to stunted growth (Blanton et al., Science 2016) and obesity (Le Chatelier et al., Nature 2013)
- ➢Promote anti-tumour immunity (Sivan et al., Science 2015)
- ➢Prevent anti-tumour immunity (Spencer et al., Science 2021)
- ➢Protect from gut infections (Fukuda et al., Nature 2011)



Arboleya et al., 2016

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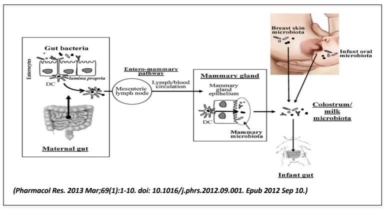
Vertical Transfer of Bifidobacteria from Mother to Neonate?

Origin of the bacteria isolated from breast milk: is there a bacterial entero-mammary pathway?



(Pharmacol Res. 2013 Mar;69(1):1-10. doi: 10.1016/j.phrs.2012.09.001. Epub 2012 Sep 10.)

The Origin of Human Milk Bacteria: Is There a Bacterial Entero-Mammary Pathway during Late Pregnancy and Lactation?[1–4]

Juan M. Rodríguez*
Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, Madrid, Spain

Article | Published: 24 February 2020

Viable bacterial colonization is highly limited in the human intestine in utero

E. Rackaityte, J. Halkias, E. M. Fukui, V. F. Mendoza, C. Hayzelden, E. D. Crawford, K. E. Fujimura, T. D. Burt & S. V. Lynch

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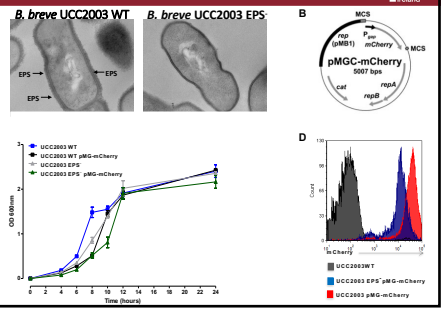
Generation of mCherry expressing *B. breve* UCC2003 to investigate Host-Microbe interactions

- *B. breve* UCC2003 was originally isolated from healthy infant stool
- Production of a surface **exopolysaccharide (EPS)**, which consists of extracellular polymerized glycans
- We have previously shown that the EPS layer has immunomodulatory effects



Fanning et al, 2012 PNAS 109 no.6: 2108-2113

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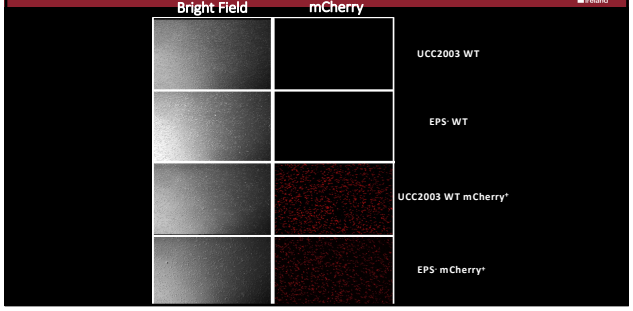
mCherry expressing *B. breve* UCC2003



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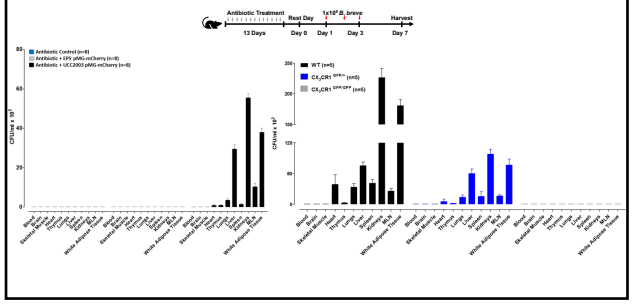
Bacterial EPS and host CX_3CR1 are required for the translocation of *B. breve* UCC2003 to systemic tissues



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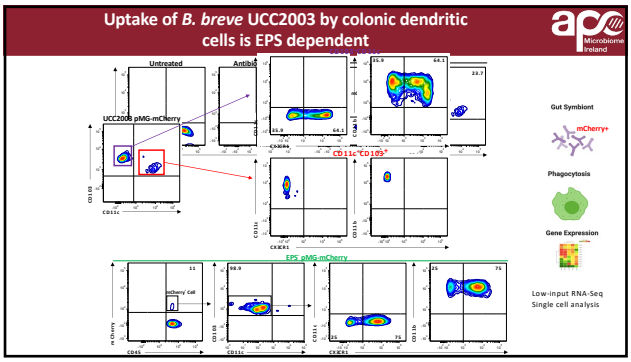
Uptake of *B. breve* UCC2003 by colonic dendritic cells is EPS dependent



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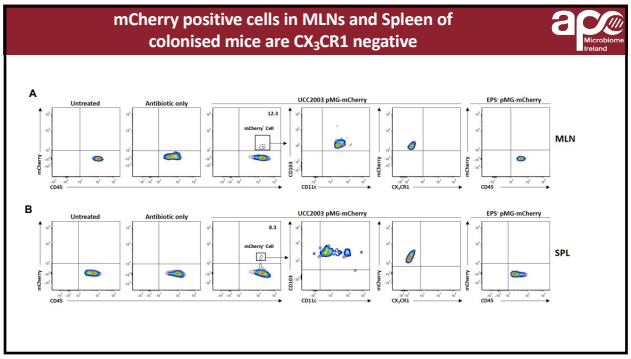
mCherry positive cells in MLNs and Spleen of colonised mice are CX_3CR1 negative



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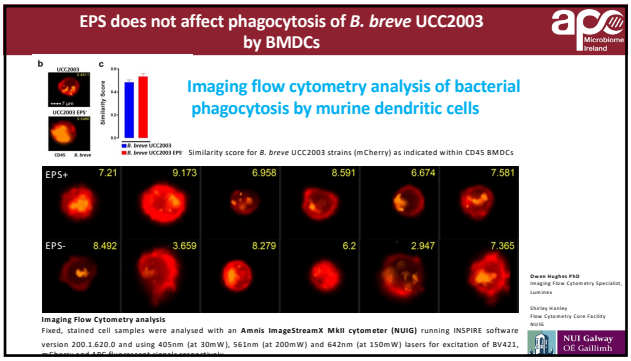
EPS does not affect phagocytosis of *B. breve* UCC2003 by BMDCs



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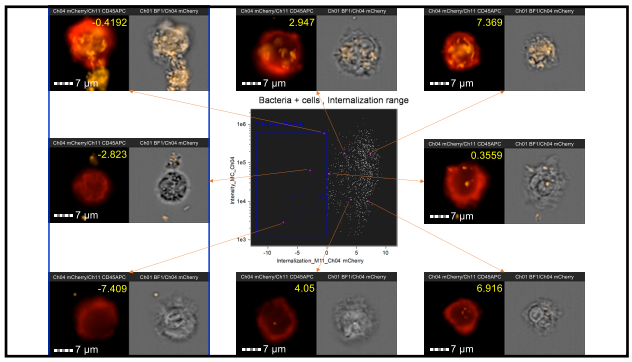

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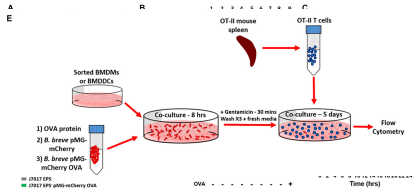
EPS blocks antigen presentation from *B. breve* to OT-II CD4⁺ T cells by BMDCs



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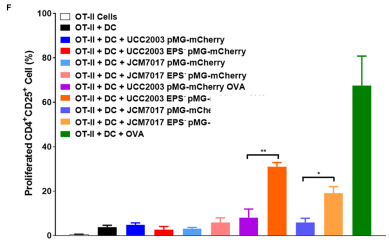
EPS blocks antigen presentation from *B. breve* to OT-II CD4⁺ T cells by BMDCs



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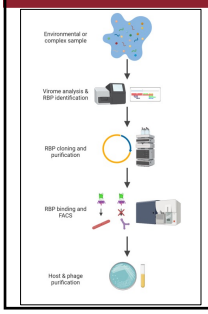
PhRACS as a discovery tool for unknown phage-bacterial host combinations



Phage **R**BP-**A**ctivated **C**ell **S**orting (PhRACS) - PhRACS

PhRACS workflow.

- Virome analysis is first performed on a complex sample, and putative Phage Receptor Binding Protein (RBP) nucleotide sequences are identified.
- RBP-encoding genes are then cloned on a suitable plasmid and expressed.
- Incubation of GFP-tagged RBPs with the processed sample then allows isolation of the phage and its bacterial host from the complex sample by FACS.

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Bacterial host retrieval from the intestinal niche using PhRACS

TABLE 3 Microbial composition (greater than or equal to 0.01% read abundance) in fecal sample T60III by phylum, family, genus, and *Bifidobacterium* species

Analysis	Level	Taxon	Abundance (%)
16S	Phylum	*Actinobacteria*	18.78
		Bacteroidetes	7.89
		Firmicutes	2.26
		Proteobacteria	71.05
16S	Family	*Bifidobacteriaceae*	18.72
		Bacteroidaceae	6.36
		Porphyromonadaceae	1.52
		Enterobacteriaceae	71.00
16S	Genus	*Bacteroides*	6.36
		Parabacteroides	1.52
		Escherichia-Shigella	70.48
		Bifidobacterium	18.72
ITS	*Bifidobacterium* species	*B. longum* subsp. *longum*	60.45
		B. pseudocatenulatum	38.51
		B. bifidum	0.89
		B. adolescentis	0.05
		B. longum subspecies	0.02
		B. pairmae	0.02
		B. longum subsp. *infantis*	0.01
		B. animalis subsp. *lactis*	0.01
		B. asteroides	0.01
		B. breve	0.01
		B. scardovii	0.01
		Other *Bifidobacterium* species	0.02

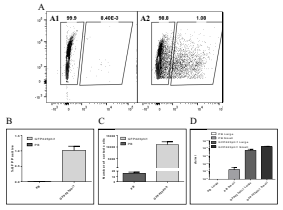

FIG 5 (A) Dot plot analysis of the T60III bifidobacterial community labeled with PB (A1) and HisGFP-Rv1423ph1 (A2). (B) Proportion of GFP-positive events expressed as a percentage of total events processed. (C) Number of events sorted using gating strategy targeting GFP-positive cells evident in Fig. 4, panel A. (D) Plate counts (on MRS agar) of populations exhibiting distinct colony morphologies present in sorted population.

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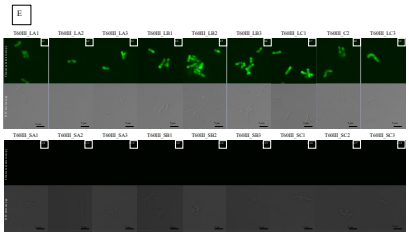
Validation of bacterial host retrieval from the intestinal niche using PhRACS



(E) Confirmation of recovery of potential host by GFP-RBP labeling of *B. pseudocatenulatum* cultures derived from isolated colonies (E1 to E9). Unlabeled co-isolates (*B. longum* subsp. *longum*) are shown in E10 to E18.

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Summary

- EPS is necessary for *B. breve* UCC2003 to disseminate in the systemic tissues of microbiota depleted mice
- CX_3CR1^+ cells play a role in the initial colonisation and in the systemic dissemination of the *B. breve* UCC2003
- EPS is required for *B. breve* UCC2003 to localize with $CD11c^+CD103^+$ dendritic cells in systemic tissues
- PhRACS is a discovery tool for fishing out unknown phage-bacterial host pairs

Bifidobacterium breve Exopolysaccharide Blocks Dendritic Cell Maturation and Activation of $CD4^+$ T Cells

Needle in a Whey-Stack: PhRACS as a Discovery Tool for Unknown Phage-Host Combinations

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