

NUTRITION, HEALTH, IMMUNESYSTEM

a simple story…..?

CLANA 2018

Stefan Jakob

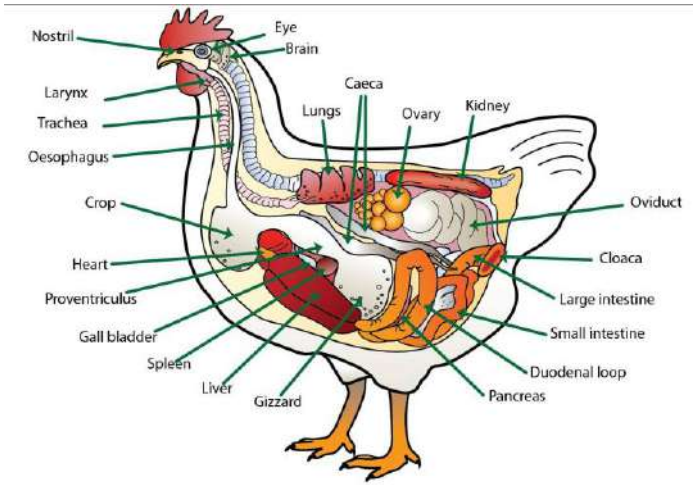


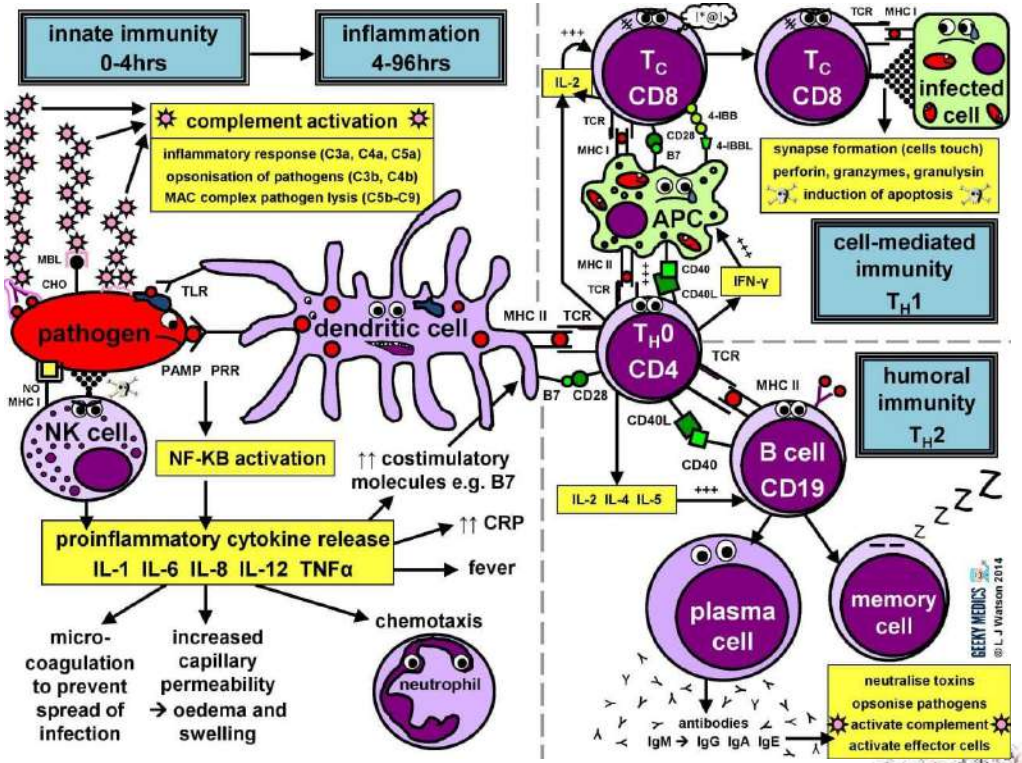


NUTRITION, HEALTH, IS

a simple story !!!!


ÖZIL
10
adidas
8


Nostril
Eye
Brain
Larynx
Trachea
Oesophagus
Caeca
Lungs
Ovary
Kidney
Crop
Oviduct
Heart
Cloaca
Proventriculus
Large intestine
Gall bladder
Small intestine
Spleen
Duodenal loop
Liver
Gizzard
Pancreas


innate immunity
0-4hrs
inflammation
4-96hrs
complement activation
inflammatory response (C3a, C4a, C5a)
opsonisation of pathogens (C3b, C4b)
MAC complex pathogen lysis (C5b-C9)
MBL
CHO
TLR
pathogen
dendritic cell
PAMP PRR
NO MHC I
NK cell
NF-KB activation
↑↑ costimulatory molecules e.g. B7
proinflammatory cytokine release
IL-1 IL-6 IL-8 IL-12 TNFα
↑↑ CRP
fever
chemotaxis
micro-coagulation to prevent spread of infection
increased capillary permeability → oedema and swelling
neutrophil
IL-2
TC CD8
TC CD8
TCR MHC I
infected cell
4-IBB
4-IBBL
CD28
B7
synapse formation (cells touch)
perforin, granzymes, granulysin
induction of apoptosis
APC
MHC II
CD40
CD40L
IFN-γ
cell-mediated immunity
TH1
MHC II TCR
TH0 CD4
TCR
B7 CD28
humoral immunity
TH2
CD40L
CD40
B cell CD19
IL-2 IL-4 IL-5
plasma cell
memory cell
antibodies
IgM → IgG IgA IgE
neutralise toxins
opsonise pathogens
activate complement
activate effector cells
GEEKY MEDICS
© L J Watson 2014

What is gut health - defintion

Gut health covers multiple positive aspects of the gastrointestinal (GI) tract, such as

- the effective digestion and absorption of food / feed
- the absence of GI illness
- normal and stable intestinal microbiota
- effective immune status
- state of well-being.

Bischoff S., 2011

What is gut health => some consequences….



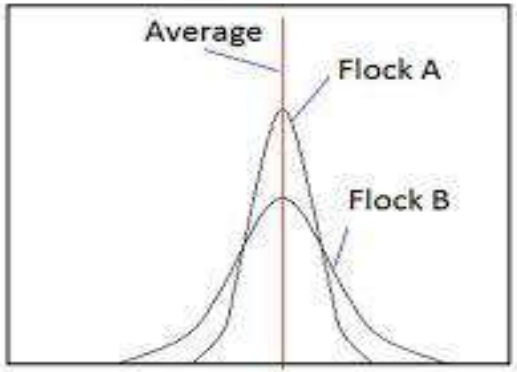
Hock burns



Foot pad lessions

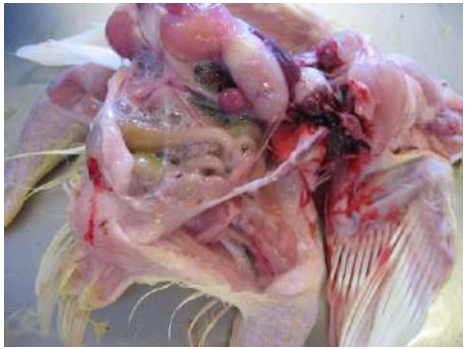


Breast blisters

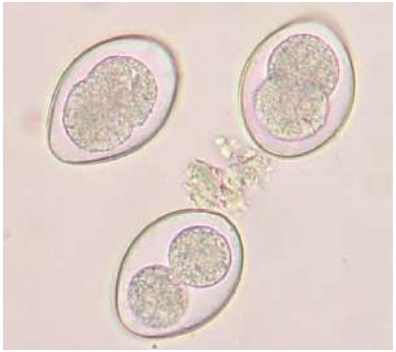


Bird Body Weights

What is gut health => some consequences….II



colibacillosis



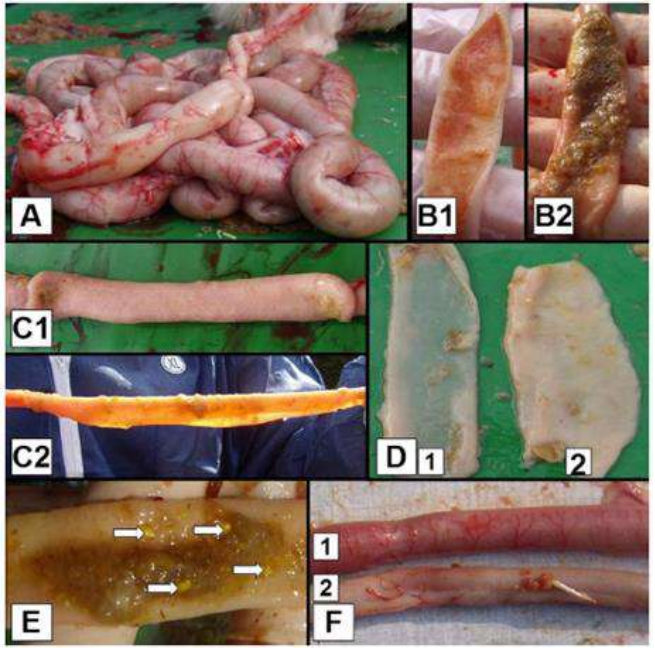
coccidiosis



c. perfringens / NE



PWD

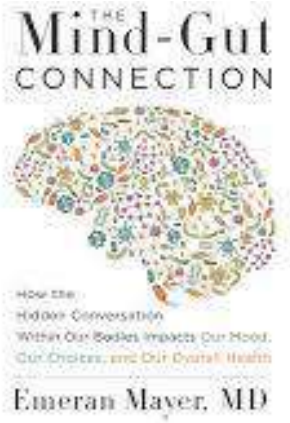


Ducatelle R., Ghent Univ.

Dysbiois

Experts view





Emeran A. Mayer, MD, PhD

Director, G. Oppenheimer Center for Neurobiology of Stress and Resilience (CNSR)

Co-Director, CURE: Digestive Diseases Research Center

Professor of Medicine, Physiology and Psychiatry

Vatche and Tamar Manoukian Division of Digestive Diseases Division of Digestive Diseases

David Geffen School of Medicine at UCLA

TED speaker

What means gut health for you ?

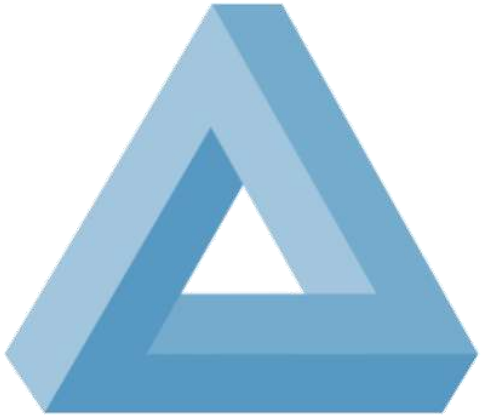
Which diseases do you encounter, as a physician ?

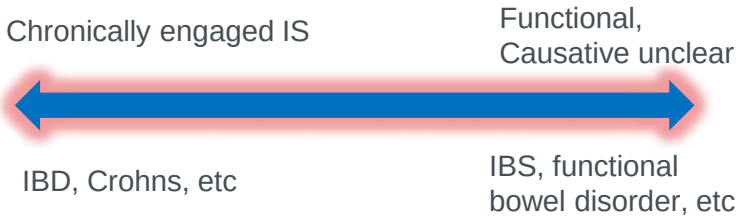
Which role does the microbiome play ?

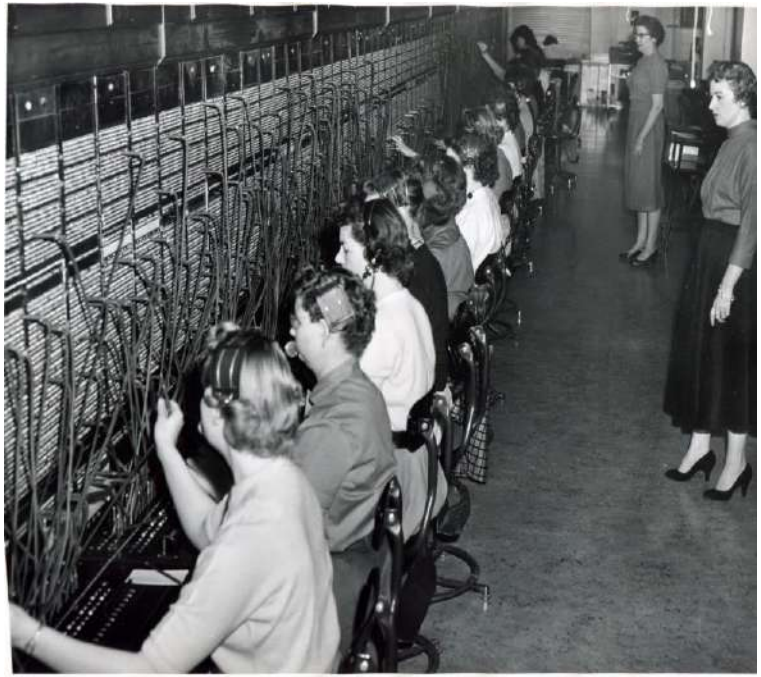
In this context, what are the « on the edge » concepts

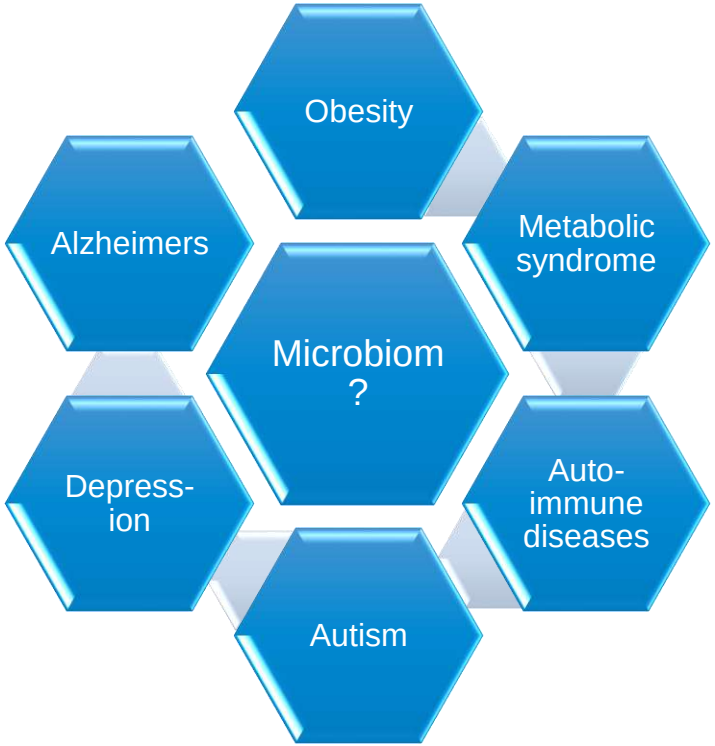
In human medicine?

Some Key Points





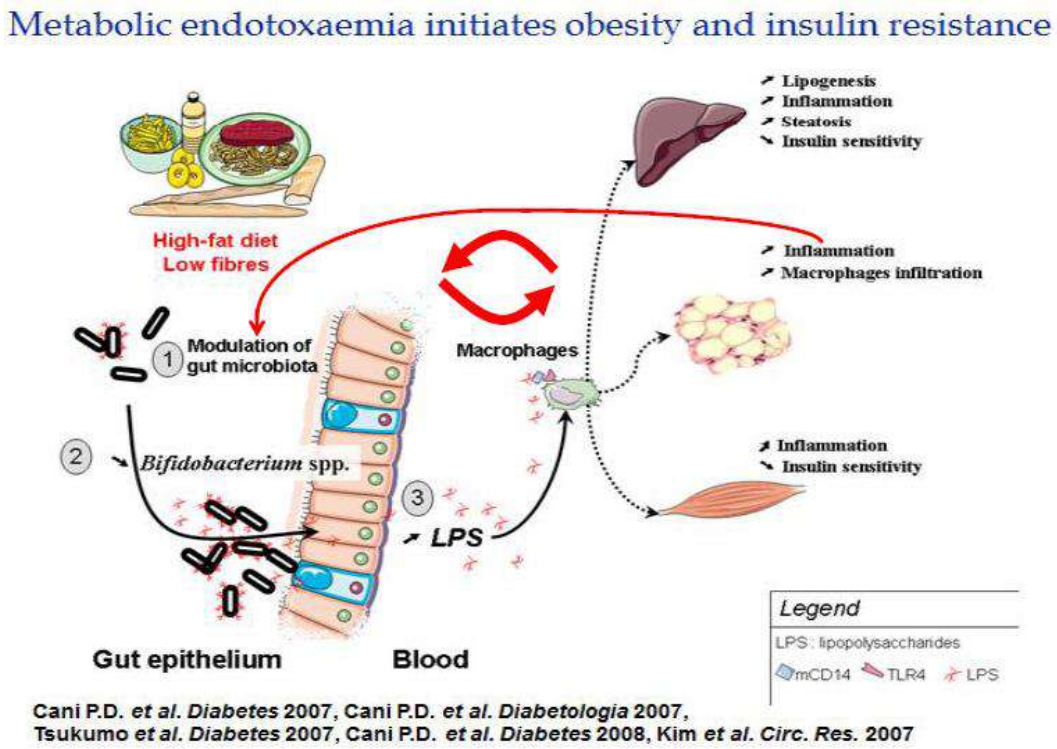




The microbiom encodes the messages the diet gives to the body

The big hope
=> the microbiota is the key to this !

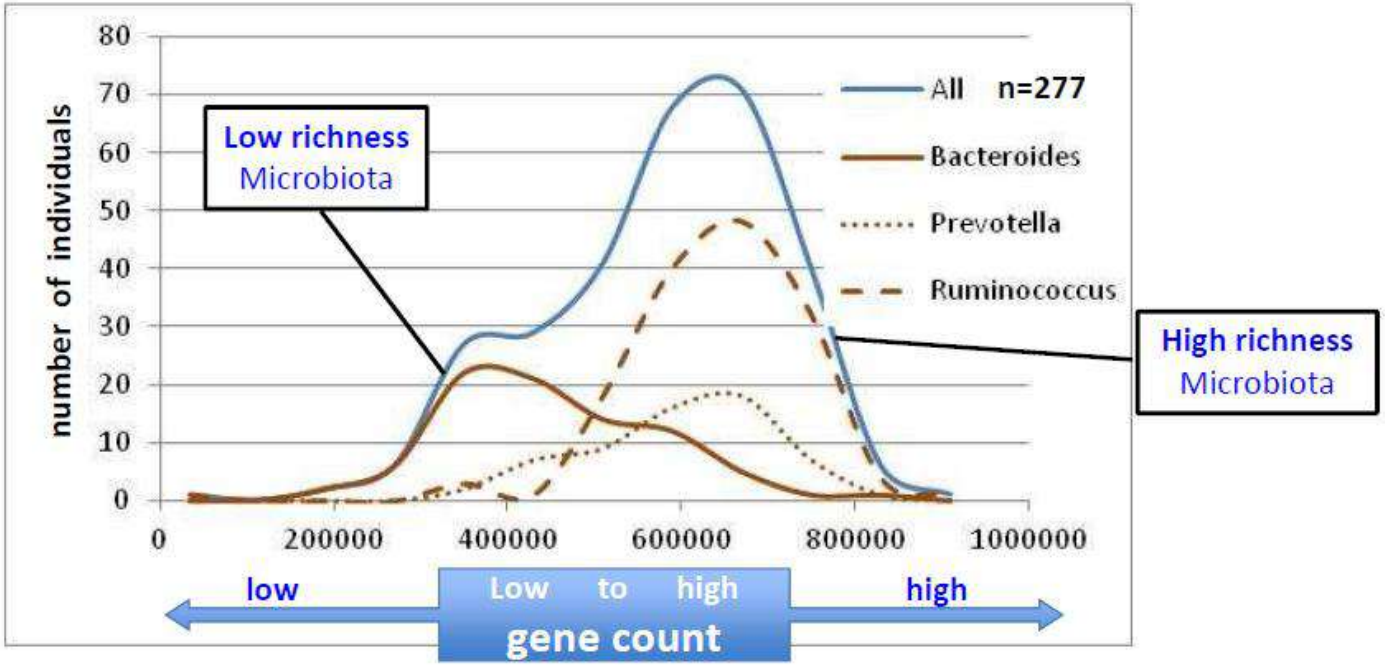
… on the way to the homo dysbioticus?



Cani P.D. *et al. Diabetes* 2007, Cani P.D. *et al. Diabetologia* 2007,
Tsukumo *et al. Diabetes* 2007, Cani P.D. *et al. Diabetes* 2008, Kim *et al. Circ. Res.* 2007

Adapted from J. Doré, INRA, France

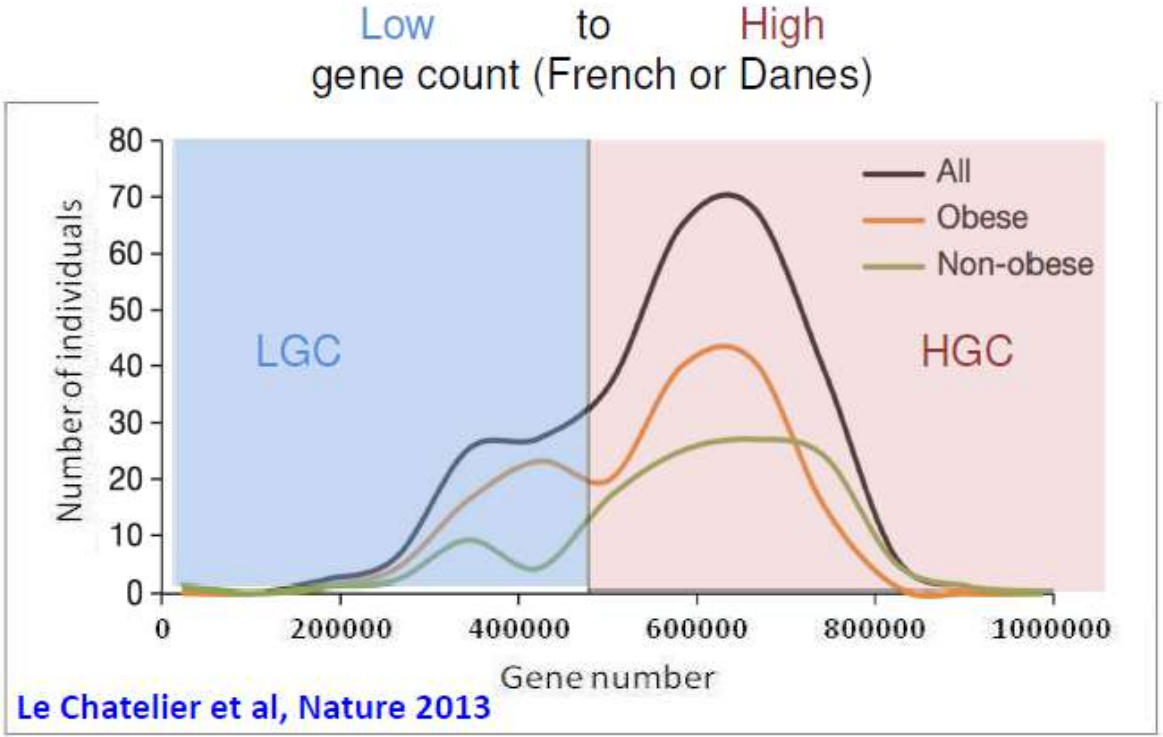
Humans: Gene Richness => Diversity: big différences



Le Chatelier *Nature* 2011

68 « species » significantly linked to gene count (richness/diversity)

Gene richness / diversity in relation to health ?



Low gene count: increased adiposity, insulin resistance, Typ II, inflammation…

Experts view

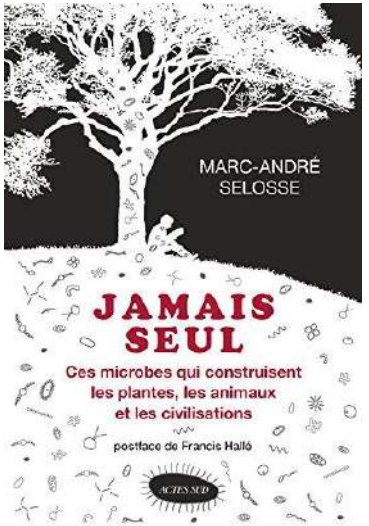


Marc-André SELOSSE, PhD

Professor at Museum national d'Histoire naturelle, Paris

at Universities of Gdansk (Poland)

and Viçosa (Brazil)






ATLAS EUROPÉEN DE LA
BIODIVERSITÉ
DES SOLS
10 000 milliards de bactéries dans l'intestin
1 000 milliards sur la peau
100 milliards ailleurs
... contre 10 000 milliards de cellules humaines

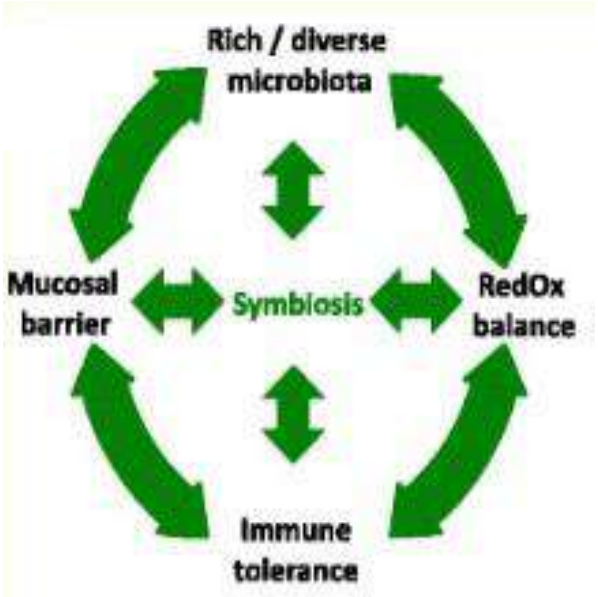
Some Key Points

- Organisms shape their environment
- Interdependence between microbiota and « living beings »

- Reducing biodiversity means trouble
  - Hygiene theory
  - Modern civilization diseases
  - In 10 years : 1 out 3 European suffers from these « modern times problems »

- If the ecosystem is disturbed, we are disturbed
- There is no steady state of the microbiota!
  - Question of resilience
  - Dynamic equilibrium

Digestive Health : The « Critical Transition » concept



Adapted from J. Doré, INRA, France

Experts view



Hervé Blottiere, PhD

Director of Research at INRA, the French National Research Institute for Agricultural and Food Research

Heads a research laboratory studying the Human Intestinal Ecosystem

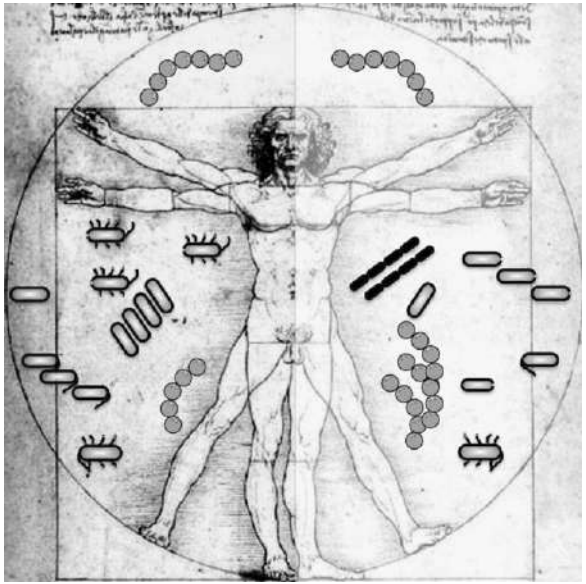
Developed a Functional Metagenomics approach to study host-microbiota cross-talk

Scientific Director of the MetaGenoPolis initiative

Expert in TV, radio and social media

Some Key Points

HOLOBIONT



Supraorgansim

Functional View is Key !!

Elrakaiby et al., 2014

PATHABIONT

PATHOBIONTS OF THE GASTROINTESTINAL TRACT

Bacterial strain	Conditions Promoting Pathogenesis
Segmented Filamentous Bacteria	• leads to colitis in SCID mice reconstituted with $CD4^{+}CD45Rb^{high}$ T cells • promotes disease in experimental models of rheumatoid arthritis and multiple sclerosis in mono-associated gnotobiotic mice
Helicobacter hepaticus	• induces colitis in C57Bl/6 *IL-10*$^{-/-}$ mice • initiates colitis and large bowel carcinoma in 129/SvEv *Rag2*$^{-/-}$ mice
Helicobacter pylori	• genetic polymorphisms of *IL1B* associated with increased risk of gastric cancer
Proteus mirabilis Klebsiella pneumonia	• responsible for inducing colitis and colorectal cancer in *T-bet*$^{-/-}$*Rag2*$^{-/-}$ (TRUC) mice
Prevotellaceae TM7	• responsible for inducing colitis in mice with mutations in the inflammasome pathway
Clostridium difficile	• can lead to pseudomembranous colitis in humans, following long-term antibiotic treatment
Vancomycin-resistant *Enterococcus*	• capable of invading the bloodstream in humans treated with broad-spectrum antibiotics

Elrakaiby et al., 2014

Normal conditions => symbiont
Ecological changes => can become pathogen

The ecosystem is shaped by microbial metabolite

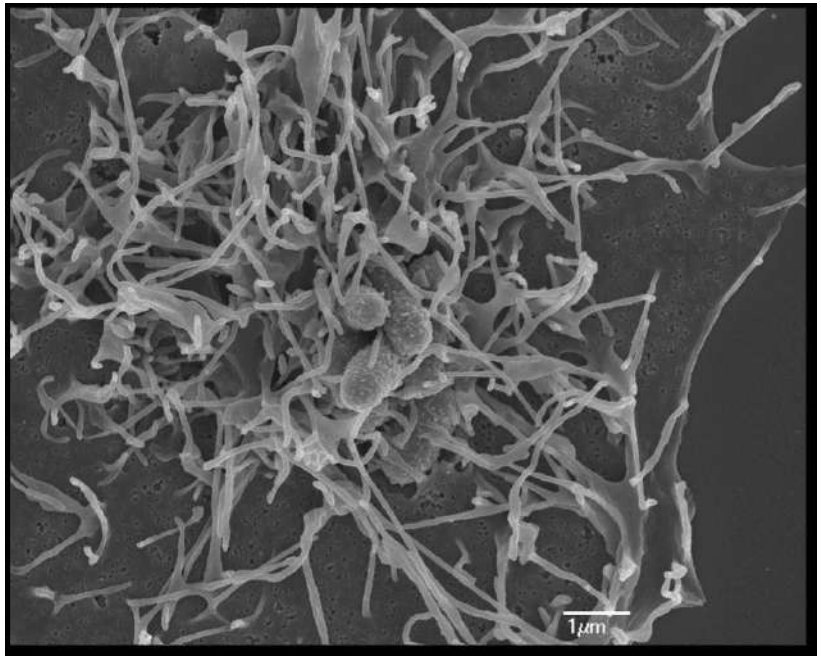
Symbiosis Gut microbiota-Host:
Holobiont concept
(the fitness of the host depends on and cannot be seen apart from its microbiota)

Microbial Ecology : microorganisms' relationships with one another and with the environment including the way they communicate.

- Cross feeding / competition for the substrate
- Quorum sensing / quorom quenching
- Competition adhesion
- Antimicrobial activities

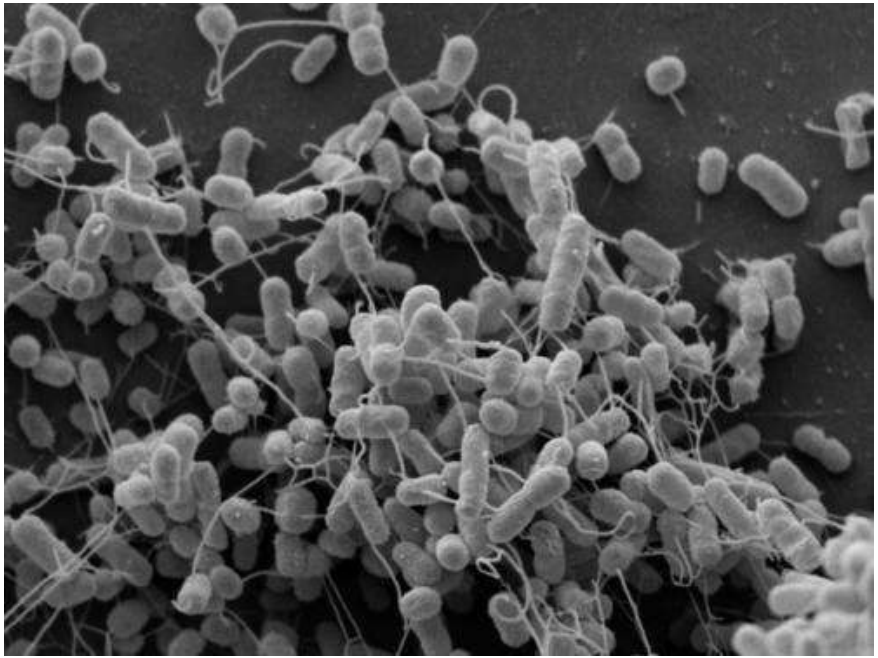
All these activities are through **metabolites** and **signals and can be harbored by probiotics**

Scanning EM of entring bacteria



Enninga, J., Inst. Pasteur, Paris

bacteria exchange amino acids via nanotubes



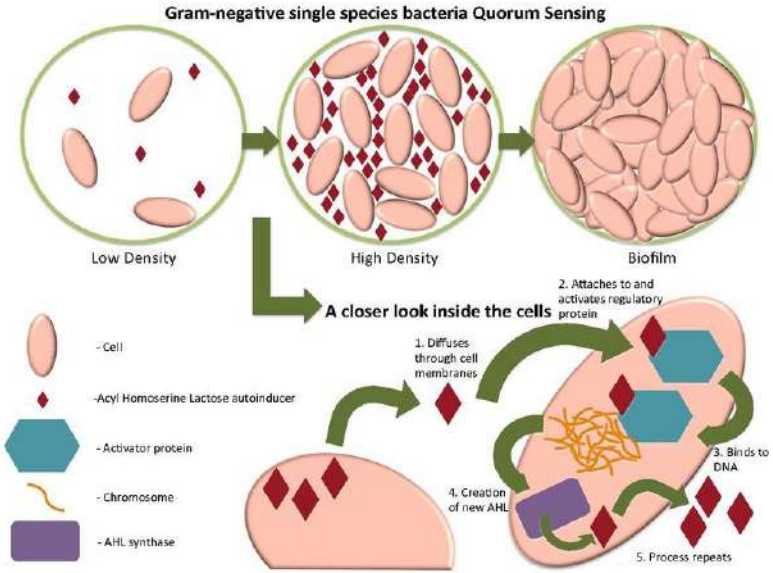
Max-Planck Inst., Jena

Quorum sensing / quorum quenching

Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density.

Quorum sensing bacteria produce and release chemical signal molecules called autoinducers that increase in concentration as a function of cell density

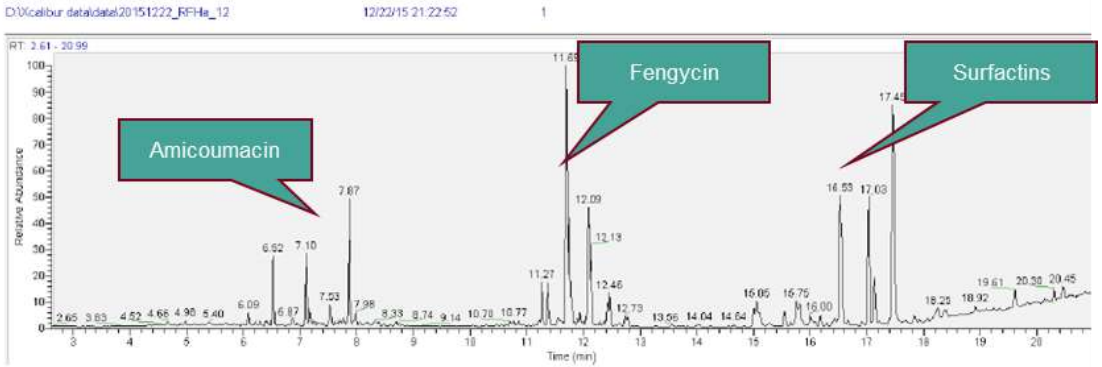
Quorum quenching is the process of preventing quorum sensing by disrupting signaling. This is achieved by inactivating signaling enzymes, by introducing molecules that mimic signaling molecules and block their receptors, or by degrading signaling molecules themselves



Bacillus species can produce anti-microbial compounds

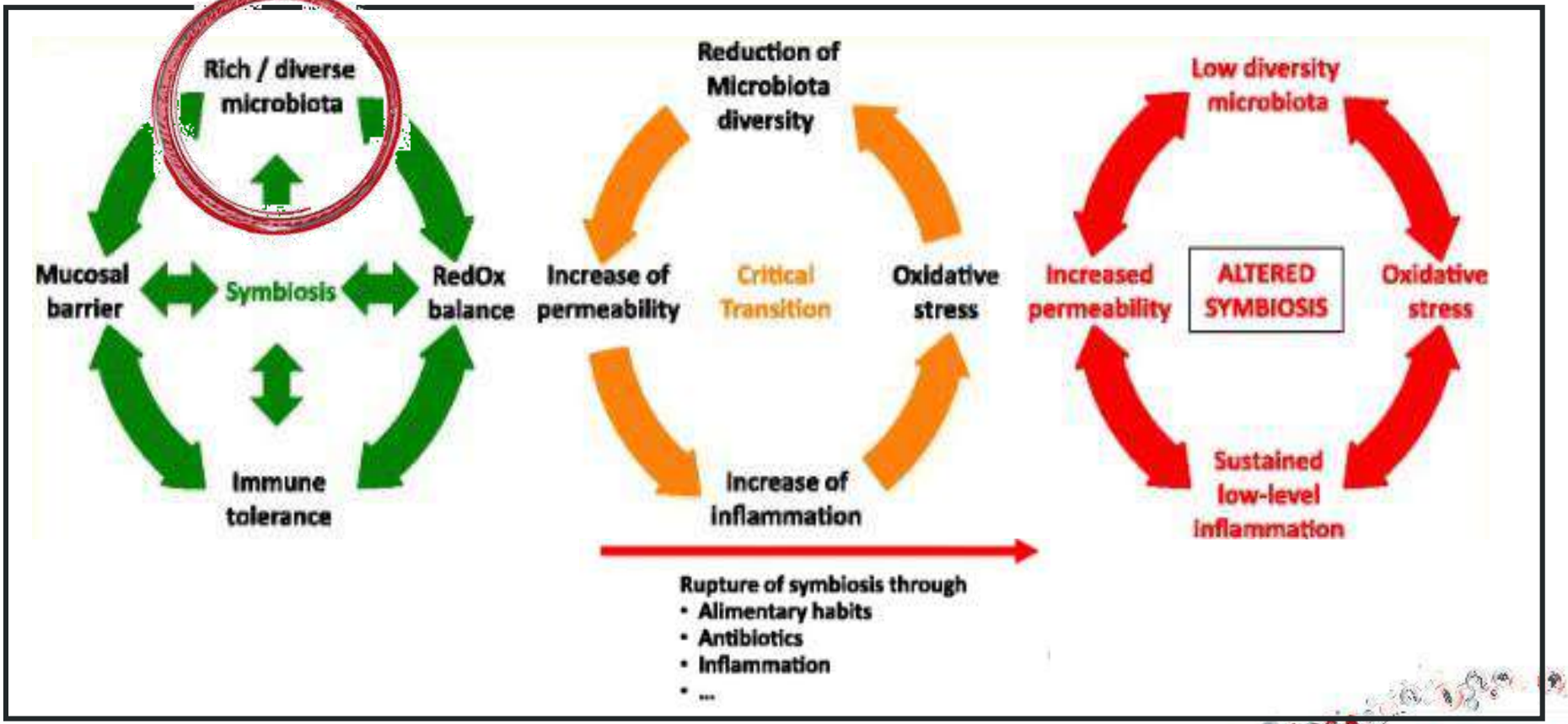
B. subtilis metabolites analysed by LCMS analyses coupled with specific search for antimicrobial and anti-inflammatory metabolites

- Around 99 peaks were identified
- Top compounds:
  - Surfactins
  - Amicoumacins
  - Lantipeptides
  - Mojavensin A, Fengysins
- No known antibiotics



These compounds will affect pathogens, but will also lead to changes to microbial ecosystem (directly or by 'emptying' of an ecological niche)

Digestive Health : The « Critical Transition » concept



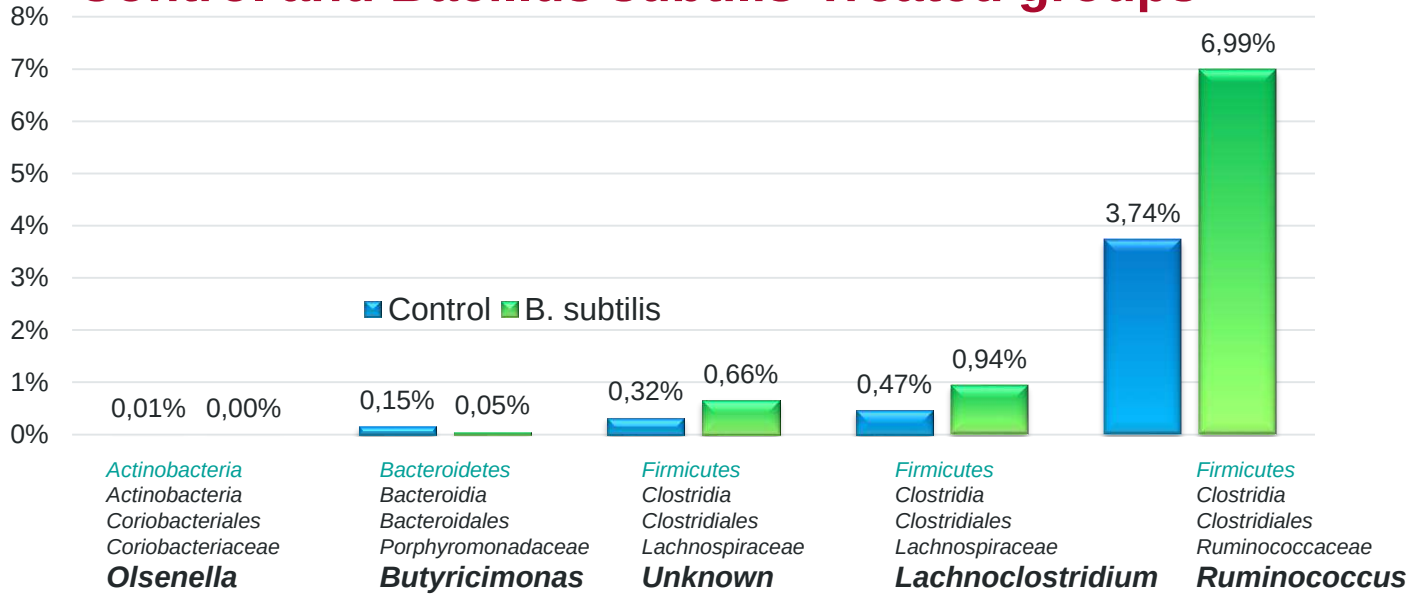
Adapted from J. Doré, INRA, France

Probiotic can modulate micobiota

- 2000 day-old male broilers (Cobb 500)
- 2 treatments (20 rep of 50 birds / treat.)
  - Control (C)
  - C + *B. subtilis* 29784 (1.10^8 CFU/kg feed)
- Measurements over 42 days
  - Feed intake (FI; g/bird)
  - Body Weight Gain (BWG; b/bird)
  - Feed Conversion Ratio (FCR)
  - Mortality rate (%)
  - 16S analyses on caecum contents at 42 days

0-42d	FI (g/bird)			BWG (g/bird)			FCR			Mortality*	
Control (C)	3570	A		1909	A		1.873	A		2.4%	A
C + *B. subtilis*	3567	A	**-0.1%**	2017	B	**+5,7%**	1.771	B	**-5.4%**	3.6%	A
p value	0,29			0,001			< 0.0001			N.S	

Only 5 genera (out of 116) changed between Control and Bacillus subtilis-Treated groups



- Main changes for *Firmicutes* phylum
  - ***Ruminococcus***, known to break down polysaccharides to oligosaccharides (Ze et al., 2013),
  - ***Lachnoclostridium*** which degrades oligosaccharides and produces SCFAs, especially butyrate (Meehan et Beiko, 2014)

Microbiota : some ‘beneficial’ bacteria depend on other bacteria for their survival – Ex Faecalibacterium prausnitzii

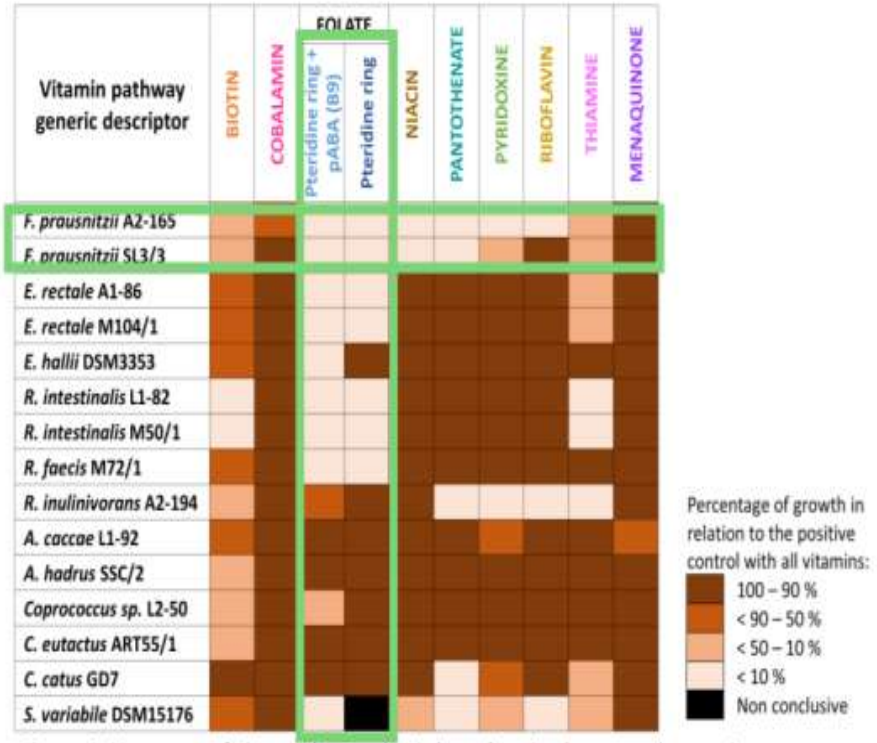


Figure 4. Heat map of vitamin biosynthesis based on *in vitro* growth experiments.

Derrien et al, 2018

F. prausnitzii uses **acetate** produced by probiotics to produce butyrate

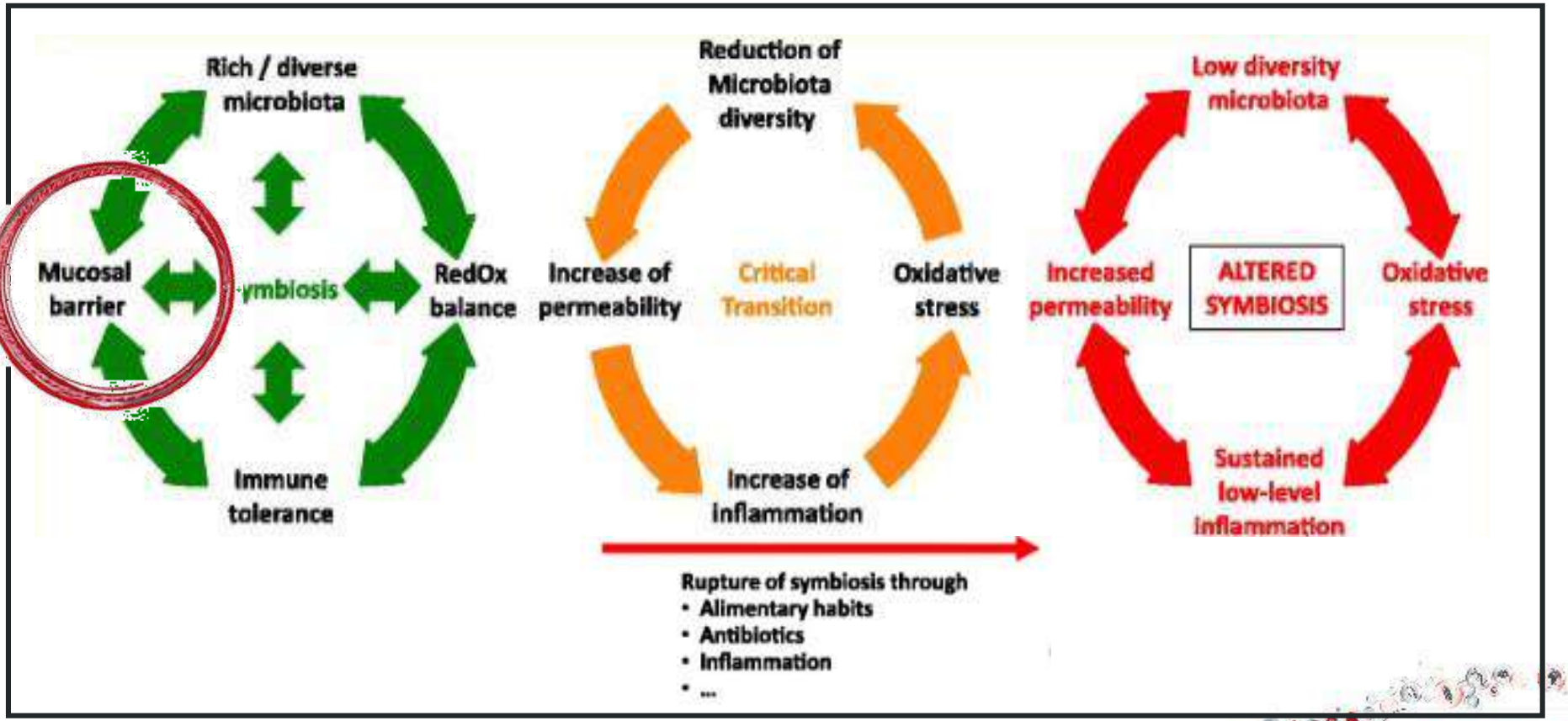
Rios-Covian, 2015

F. prausnitzii for instance exhibits high needs in **vitamins**

Vitamins can be produced by probiotics

- Folate produced by some bifidobacteria
- Riboflavin produced by some bacilli, lactococci

Digestive Health : The « Critical Transition » concept

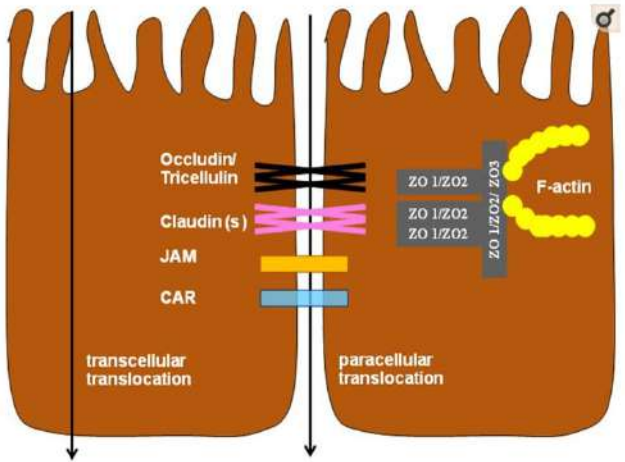


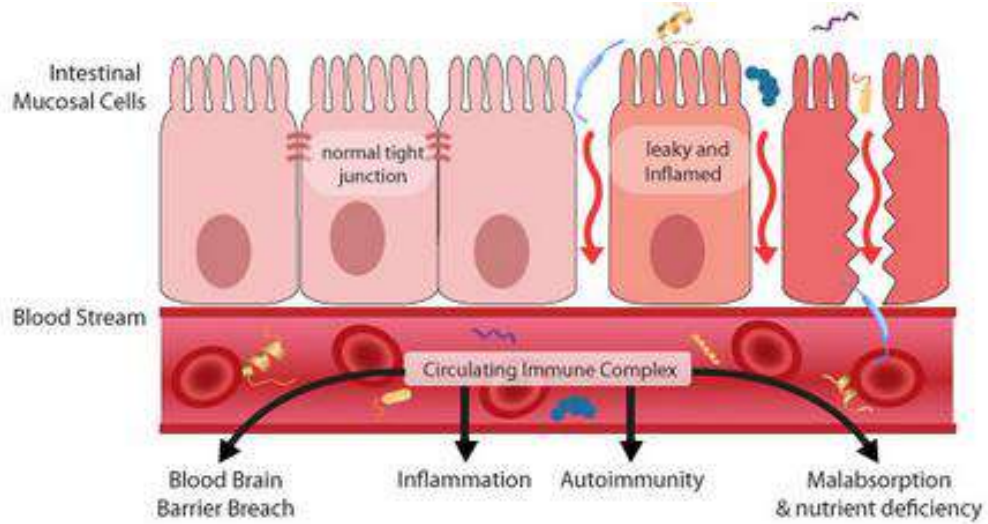
Adapted from J. Doré, INRA, France

The intestinal barrier and inflammation – leaky gut syndrom

GIT : numerous function including effective absorption – key role as a barrier against pathogens, toxins…

➔ Integrity and repair for protection of animals against disease and optimal production performance are crucial



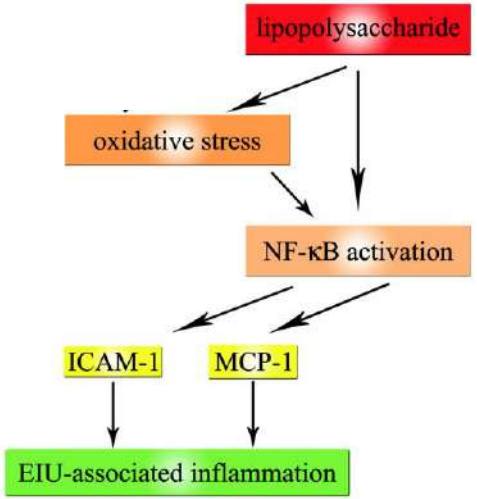


Probiotics can improve barrier function by optimizing intestinal permeability, strenghtening TJ, modulating mucin production

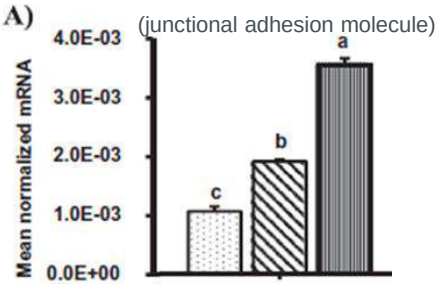
TJ and mucus layer can be reinforced

Dietary *Bacillus subtilis*-based direct-fed microbials alleviate LPS-induced intestinal immunological stress and improve intestinal barrier gene expression in commercial broiler chickens

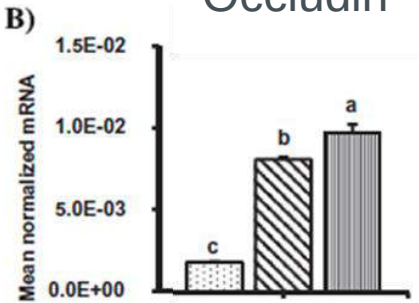
Ujvala Deepthi Gadde[a,1], Sungtaek Oh[a,1], Youngsub Lee[a], Ellen Davis[b], Noah Zimmerman[b], Tom Rehberger[b], Hyun Soon Lillehoj[a,*]



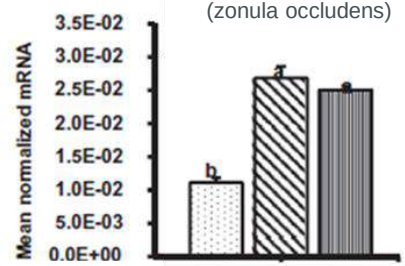
JAM-2
(junctional adhesion molecule)



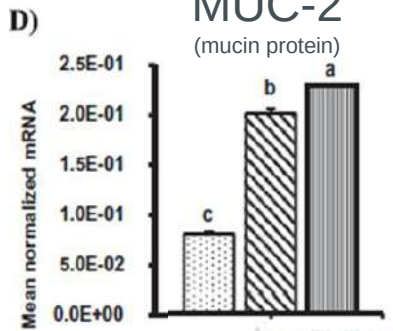
Occludin



ZO1
(zonula occludens)



MUC-2
(mucin protein)



Control Antibiotic Probiotic

Probiotics maintain intestinal barrier as measured by TEE

Transepithelial resistance (TEER) widely accepted quantitative technique to measure the integrity of tight junction dynamics in cell culture models

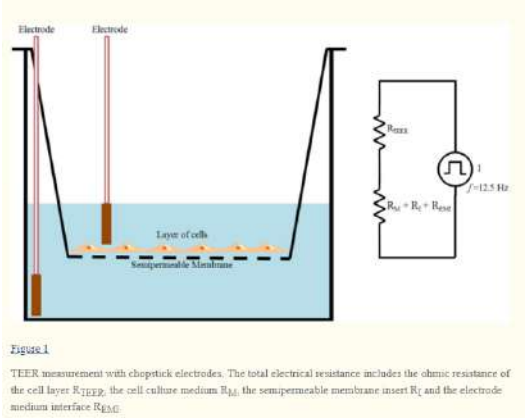


Figure 1

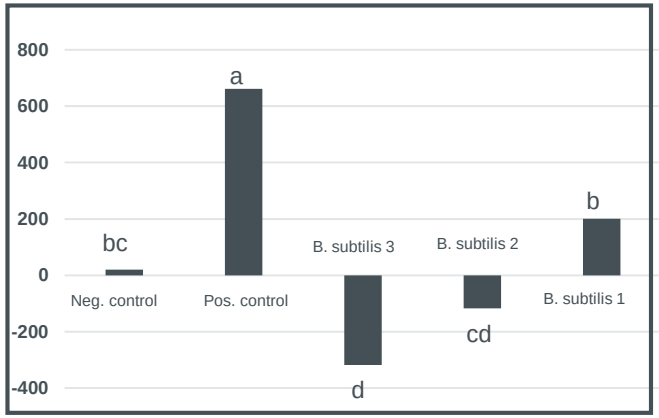
TEER measurement with chopstick electrodes. The total electrical resistance includes the ohmic resistance of the cell layer R_{TEER}, the cell culture medium R_M, the semipermeable membrane insert R_I and the electrode medium interface R_{EMI}

Model: Caco-2 cells (monolayer on transwell membrane)

Pro-inflammatory stimuli: IL1-β, flagella, DON
Use of a positive control (anti-inflammatory compound, EGCG)

Test of different Bacillus-based probiotics, including Alterion (Bacillus subtilis DSM 29784)

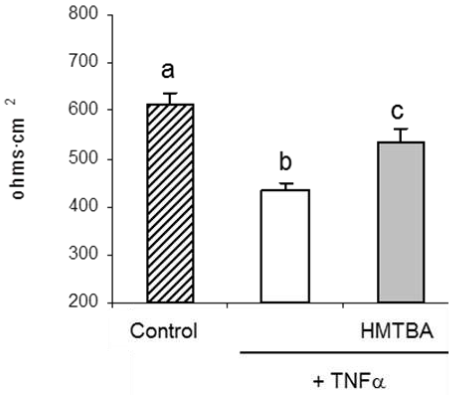
Gut integrity as measured by TEER (IL1 stimulation)

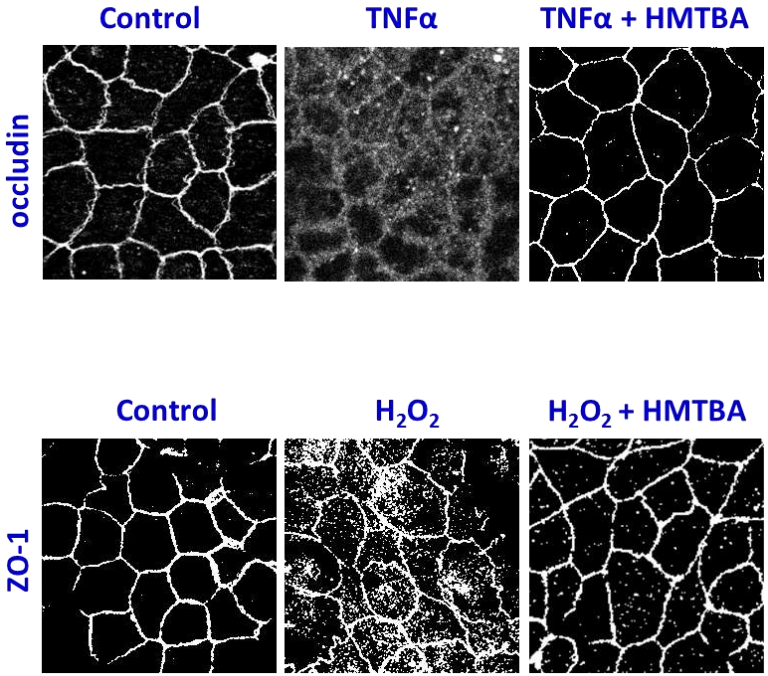


B. subtilis 29784 (1) can enhance TEER in conditions of various stimuli

Effects of probiotics are strain specific

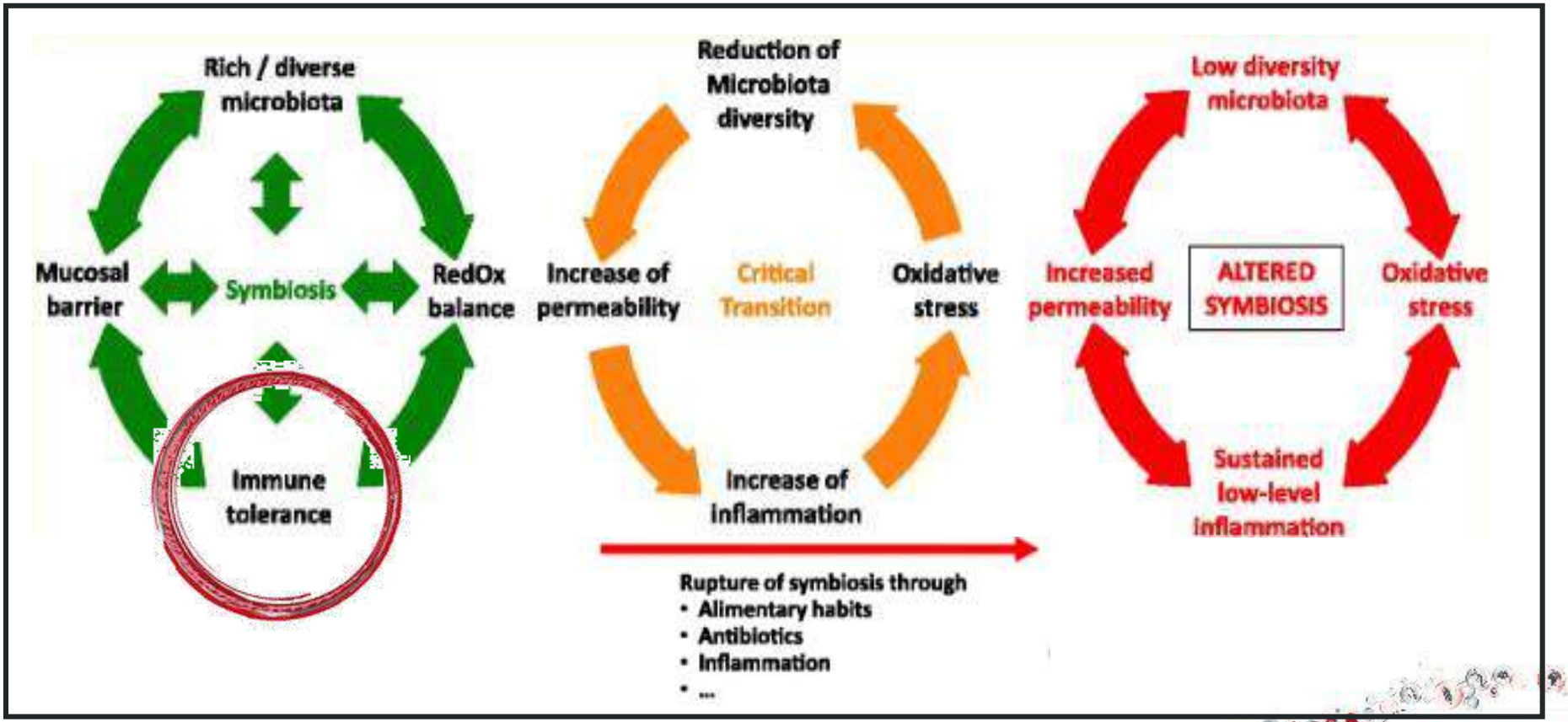
Tight junctions can be regulated by components of the diet





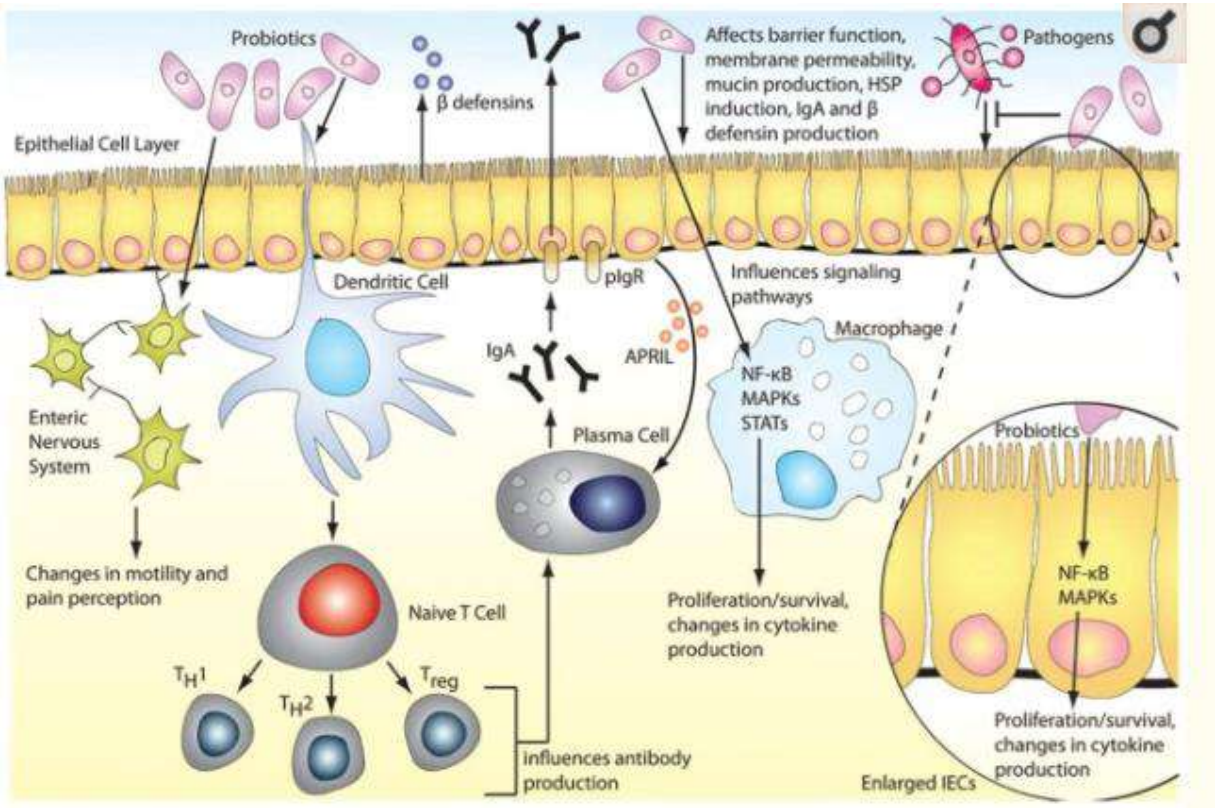
Martín-Venegas et al., 2013

Digestive Health : The « Critical Transition » concept



Adapted from J. Doré, INRA, France

Digestive Health : Impacting the IS with probiotics



Hemarajata & Versalovic, ., 2014

Probiotics can improve production IgA

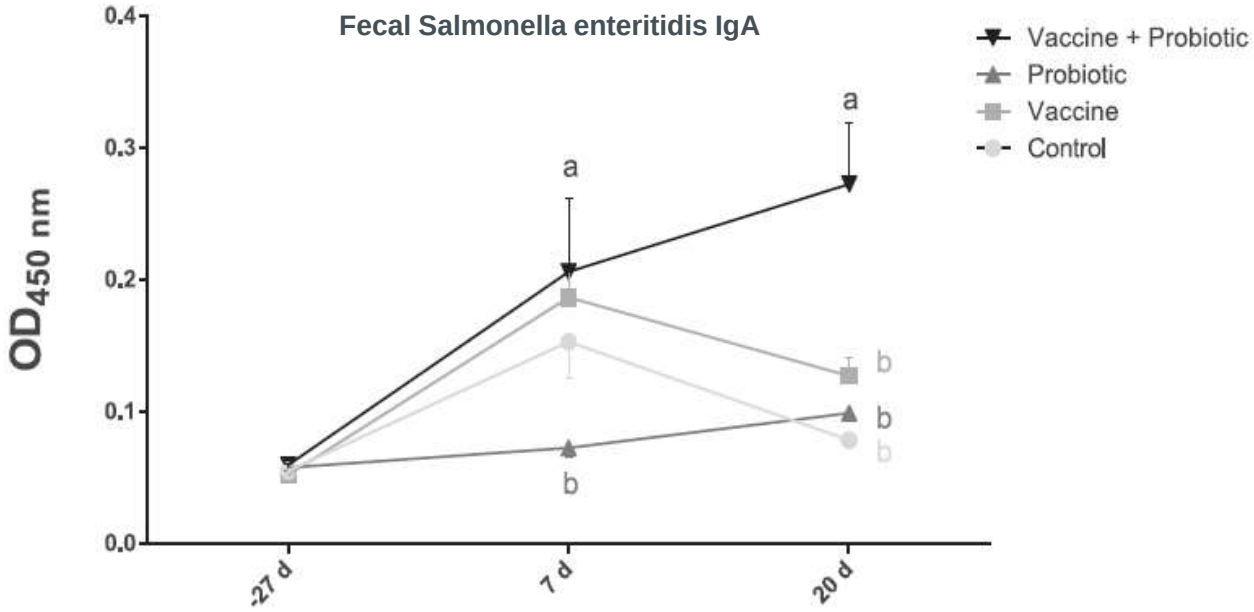
Short Communication

Effect of an *Enterococcus faecium* probiotic on specific IgA following live *Salmonella* Enteritidis vaccination of layer chickens

Breno C. B. Beirão, Max Ingberman, Celso Fávaro Jr., Dany Mesa, Leticia C. Bittencourt, Vitor B. Fascina & ...show all

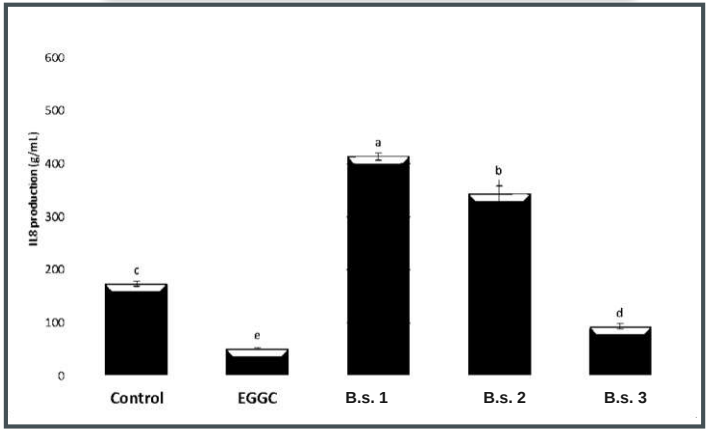
Pages 325-333 | Received 14 Dec 2017, Accepted 02 Mar 2018, Accepted author version posted online: 14 Mar 2018, Published online: 06 Apr 2018

Download citation https://doi.org/10.1080/03079457.2018.1450487



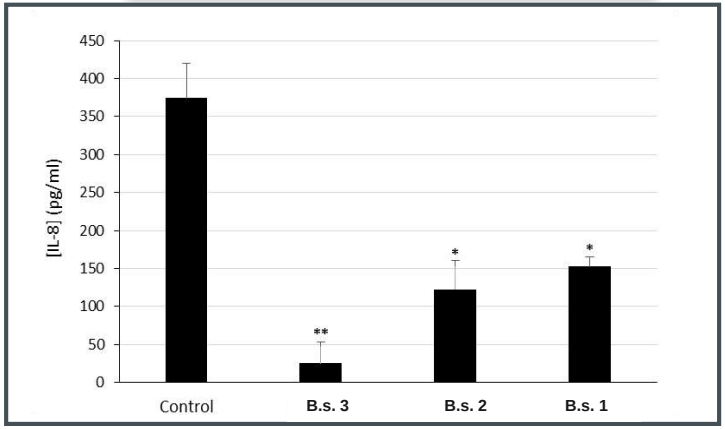
Probiotics used in poultry can improve inflammatory status

Inflammation as measured by IL8 production (DON stimulation)



Pre-treatment of Caco2 cells with B. subtilis 29784 limits inflammation after subsequent addition of different stimuli (IL1, Fla, Don)

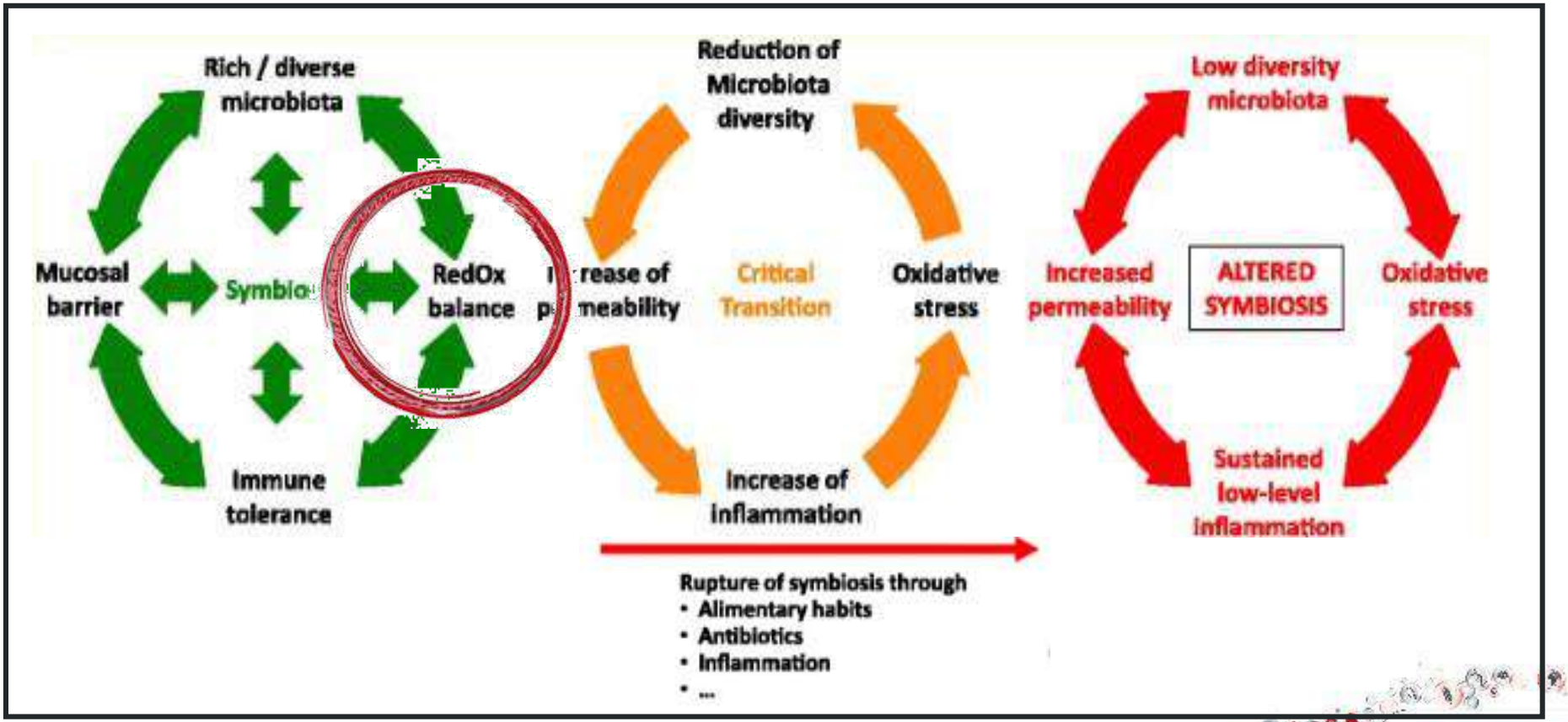
Inflammation as measured by IL8 production (IL1 stimulation)



Effects due to the cell-associated fractions as well as soluble fractions

Mechanism : B. subtilis 29784 limits NFkB activation and reduces upregulation of iNOS

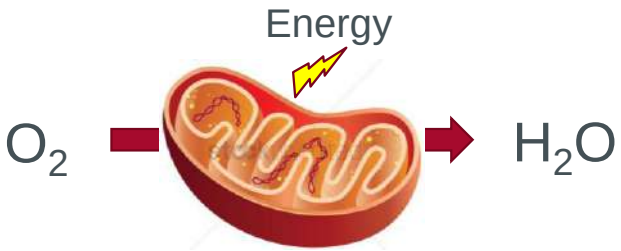
Digestive Health : The « Critical Transition » concept



Adapted from J. Doré, INRA, France

Oxidative stress and Redox mechanisms: origins

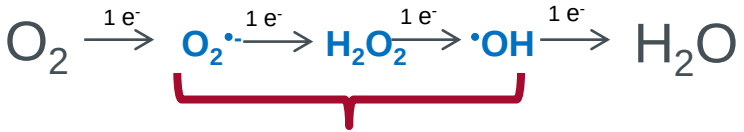
Oxygen: The fuel for life



Mitochondria

Oxygen – Oxidation

An electron (e^-) transfer story



Reactive Oxygen Species (ROS)

ROS: good or bad ?

Oxidative stress and Redox mechanisms: origins

ROS

- Free radicals
  $O_2^{\bullet-}$, $RO_2^{\bullet}$, $^{\bullet}OH$, $NO^{\bullet}$
- Nonradical oxidants
  H_2O_2, oxidized lipid or protein, Disulfides
- Redox enzymes

Redox homeostasis

Antioxidant systems

- Radical scavengers:
  CAT, SOD, Vit C-E
- Reducing systems
  Redox enzymes
  Cofactor systems
  (GSH; Trx; NADPH)

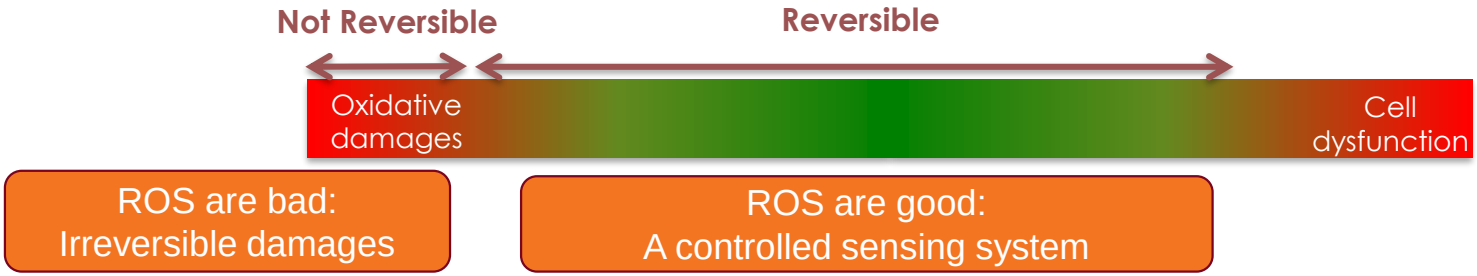


Not Reversible

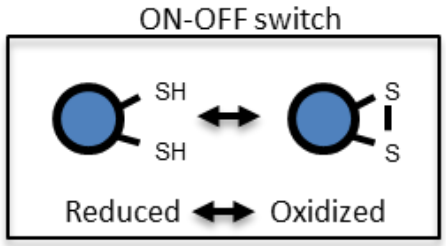
Reversible

Oxidative damages → Cell dysfunction

Oxidative stress and Redox mechanisms: origins



The **S**ulfur-> cysteine -> **SH** ->Thiol system:



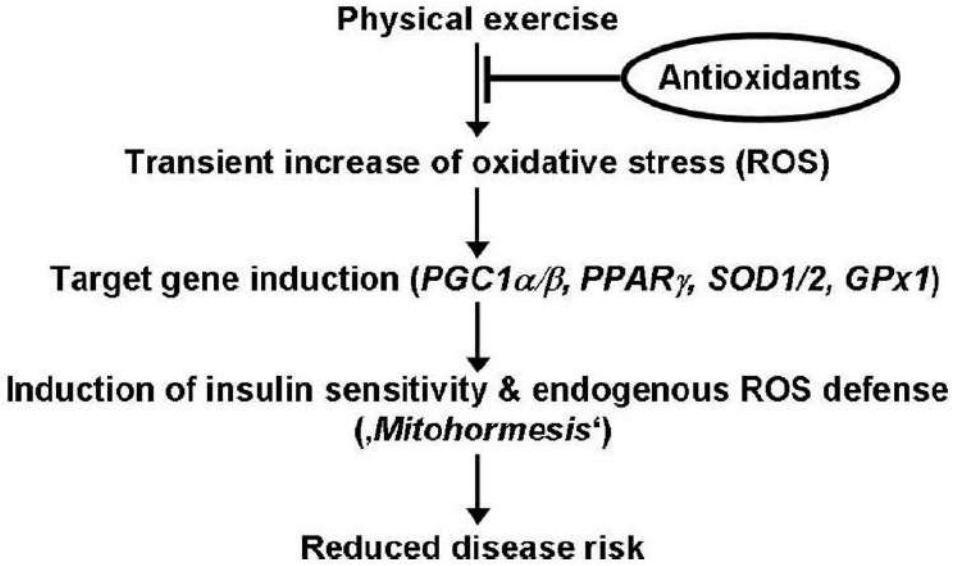


The ROS nitric oxide (NO°) act on muscle relaxation:

-Relax muscle,

-Hypertention reduction, blood pressure,

Oxidative stress and Redox mechanisms: the sports example



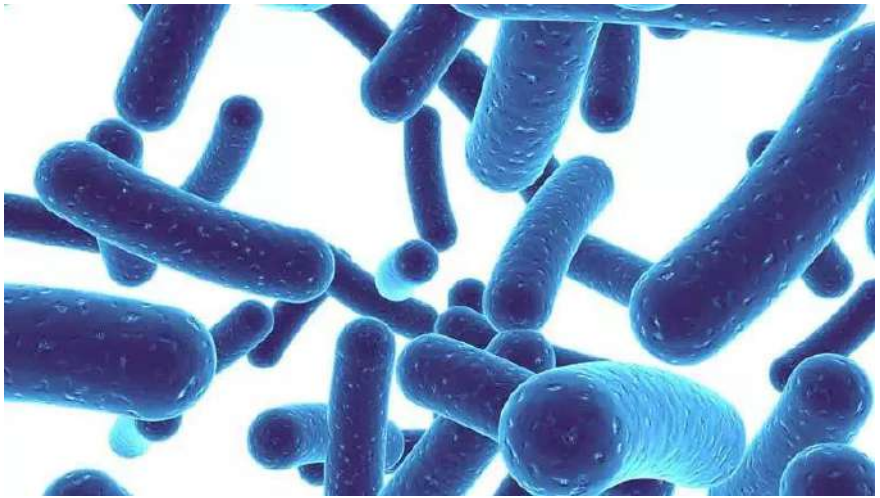


Ristow et al., 2009

Oxidative stress and Redox mechanisms: the probiotics example

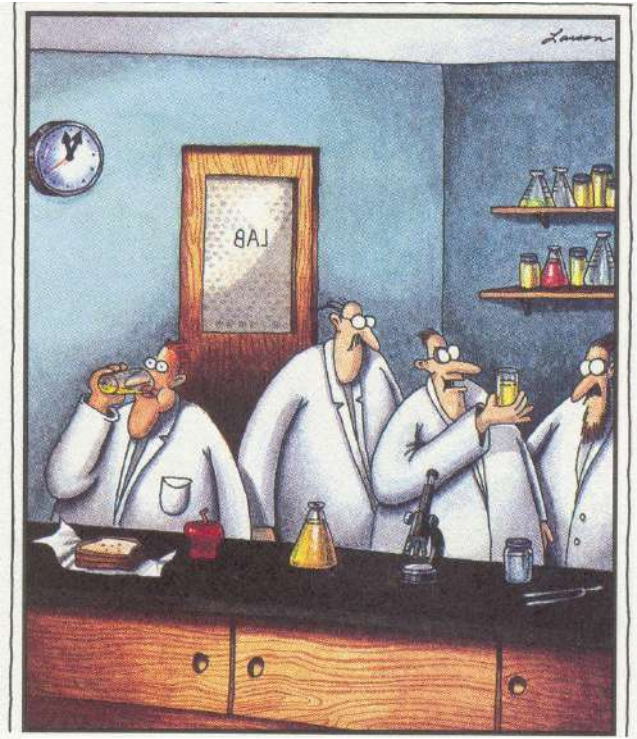
- Modulate microbiota
- Regulate signaling pathway
- Up-regulate production of host ‘antioxidases’
- Increase levels of AOX produced by host
- Down-regulate enzymes responsible of ROS production

- Chelate metal ions
- Produce ‘antioxidases’
- Produce AOX metabolites (ex: vitamins)

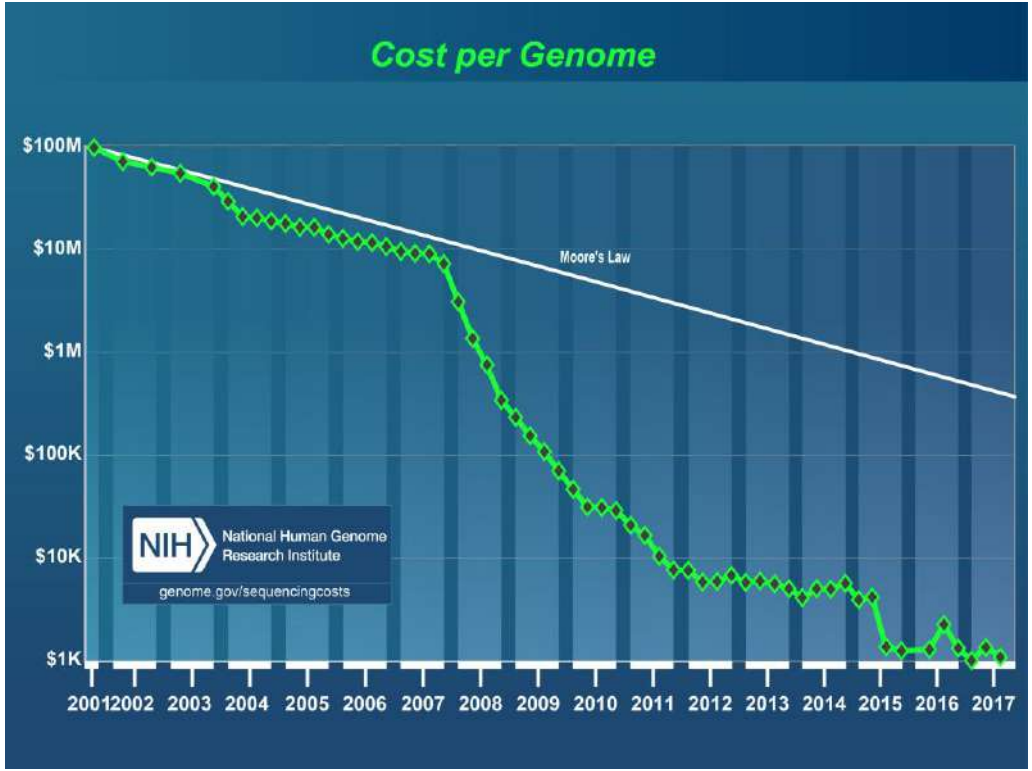


Wang et al. 2017

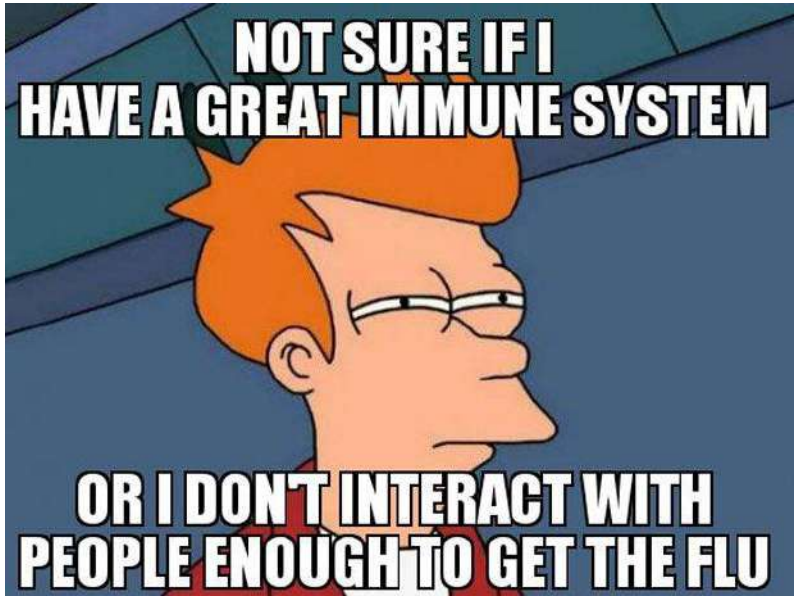
Some thoughts……

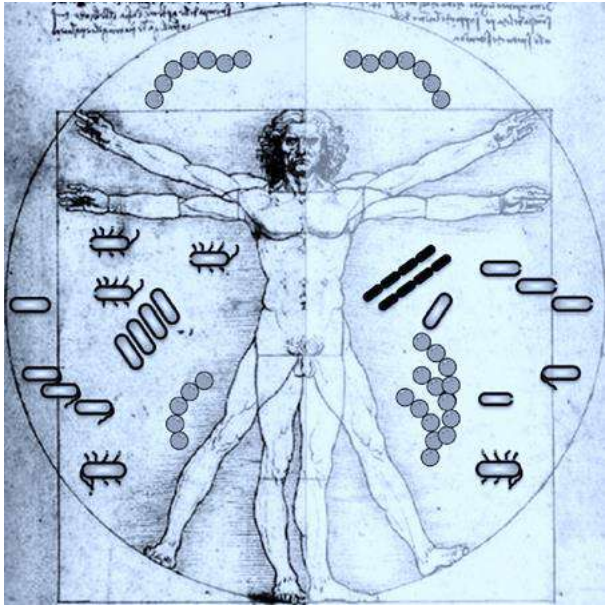


What the…? This is lemonade! Where is my salmonella culture?



Some thoughts……





FEED THE HOLOBIONT !






ARRIVEE