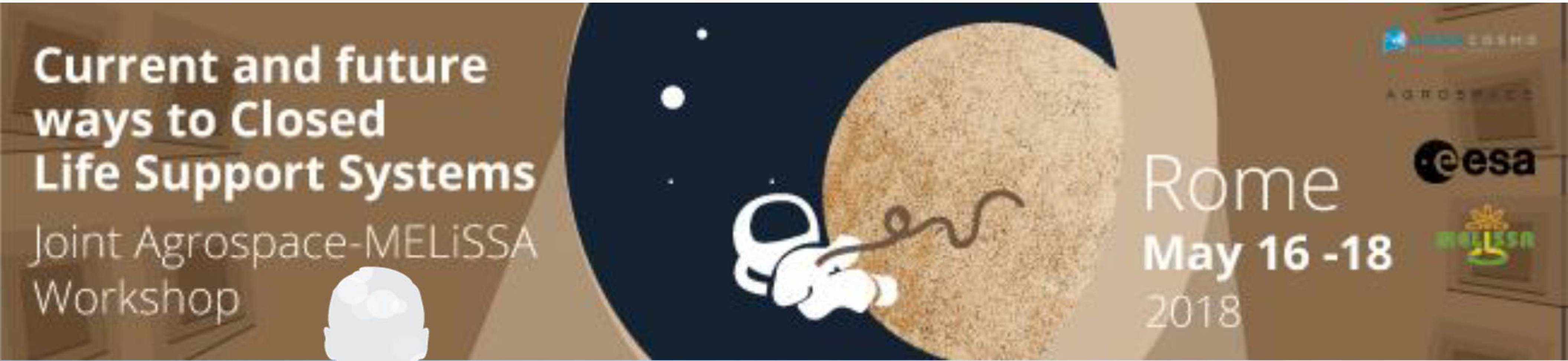
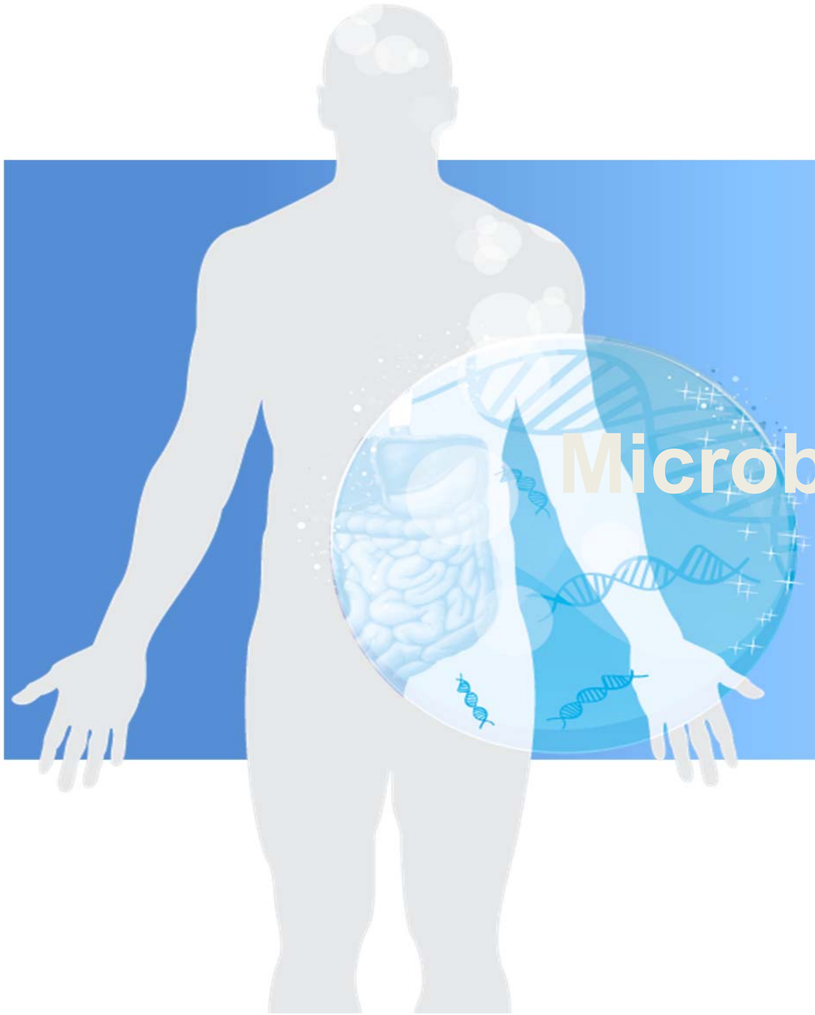


A metagenomic scan of the human intestinal microbiota

Joël Doré

Micalis & MetaGenoPolis, INRA Jouy-en-Josas, France



Homo sapiens symbiosus

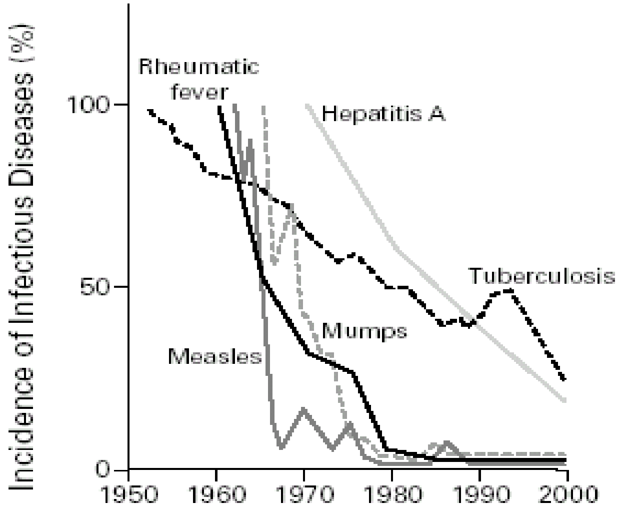
Metagenomic highlights

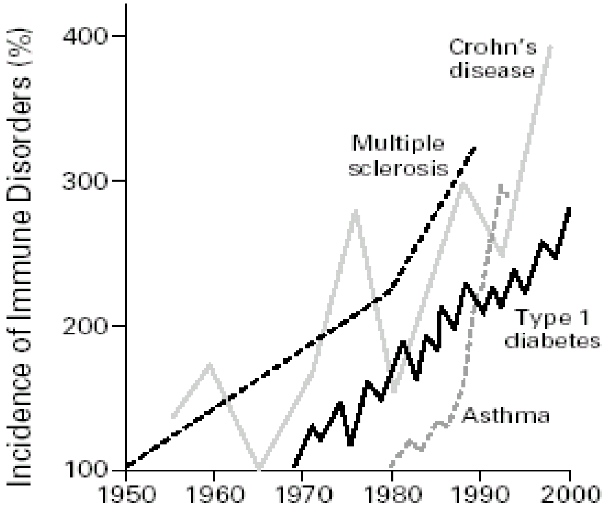
Microbiome richness as stratifier

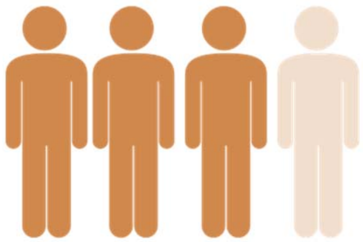
Symbiosis restauration

In spite of considerable progress in medicine …

Chronic diseases have been rising in incidence, uncontrolled, for over 60 years…







1 human in 4
by 2025

… prevention is an urgent need

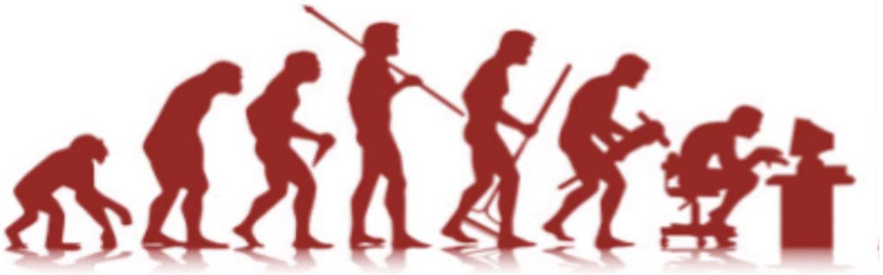
Bach JF, N Eng J Med 2002

What did we neglect ?

In the course of recent human evolution…

We drastically changed …

- ✓ Birth mode and environment
- ✓ Nutrition & physical activity
- ✓ Exposure to xenobiotics



Neglecting we are microbial …

- ✓ > 50% of our ‘cells’ are bacteria
- ✓ > 1 kg microbial biomass
- ✓ 70% unknown (yet uncultured)

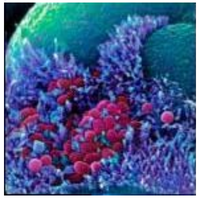
Homo sapiens ‘symbioticus’

A host-microbes symbiosis that starts at birth

adapted from Gonzalez et al. 2011, EMBO reports

Immune maturation

&



development of the microbiota

‘unique’ symbiosis : microbiota being recognized as a component of ‘self’

Maintained symbiosis : health and well-being

Disruption of ecological balance : Loss of barrier function

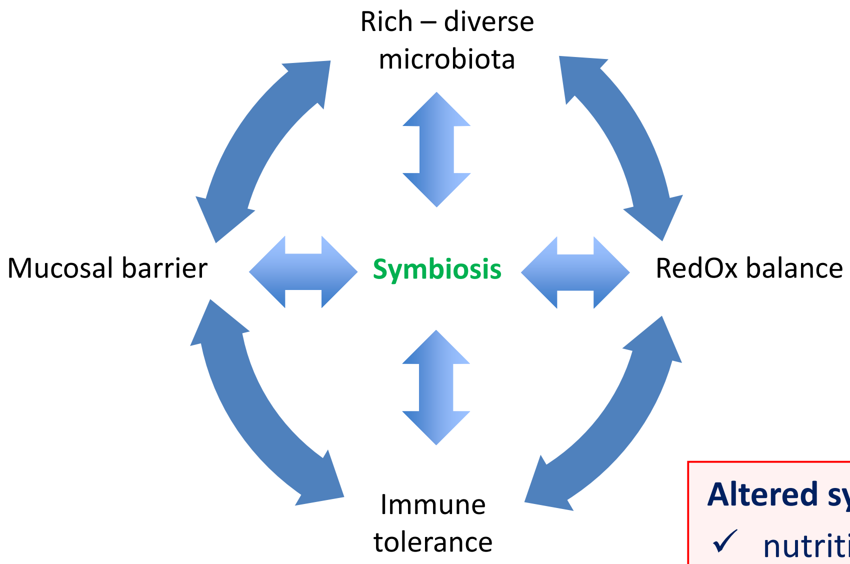
Disruption of tolerance : Immune hyper-reactivity

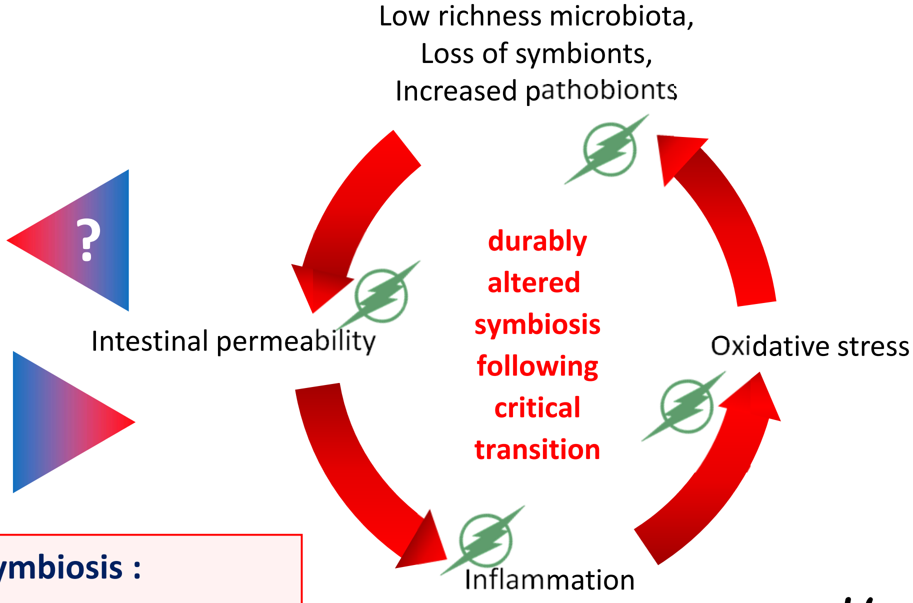
- ✓ Trophic functions
- ✓ bolic contribution

understand, monitor, fine-tune

Dysbiosis as altered man-microbes symbiosis - novel concept integrating recent observations -

The concept of critical transition* in dysbiosis





Altered symbiosis :

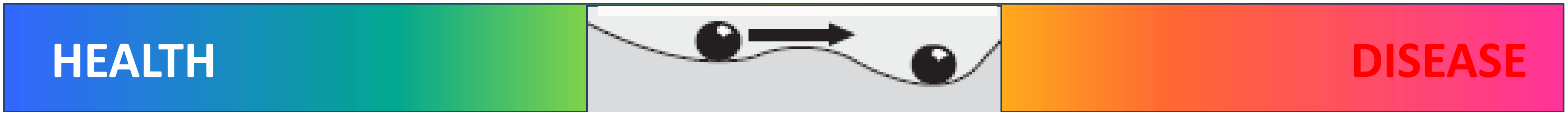
- ✓ nutrition
- ✓ Antibiotics & xenobiotics
- ✓ radio/chemotherapy
- ✓ Immuno-bilologics
- ✓ **... microgravity**
- ✓ **... radiation**

Homo symbiosus

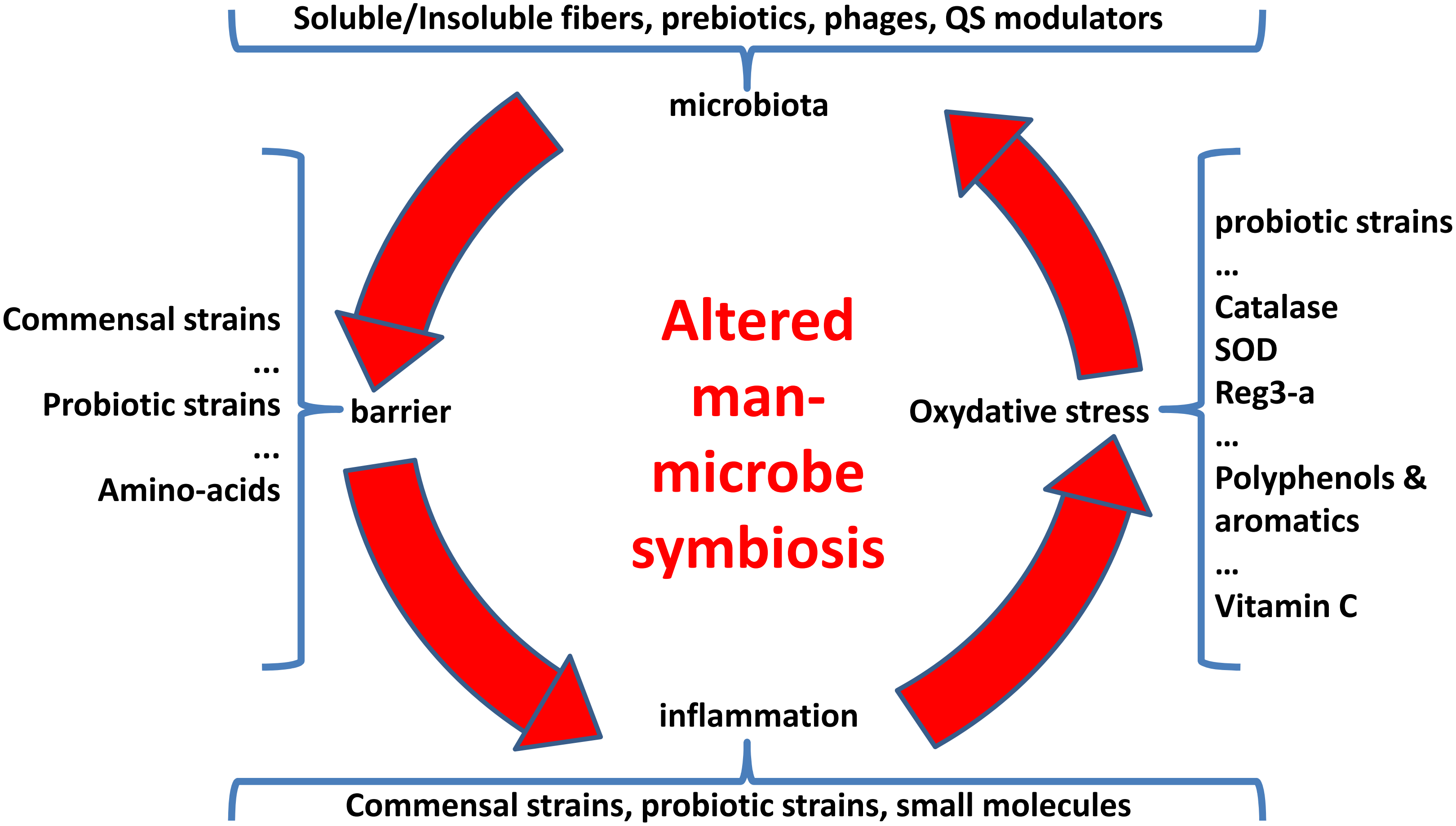
Four targets for monitoring, prevention and therapy

Van de Guchte, Blottiere, Doré 2018

*** Scheffer 2001, Kefi 2014**



Circular causalities with impact on
Prevention of alteration and symbiosis restauration.



Soluble/Insoluble fibers, prebiotics, phages, QS modulators

microbiota

Commensal strains
…
Probiotic strains
…
Amino-acids

barrier

Altered man-microbe symbiosis

Oxydative stress

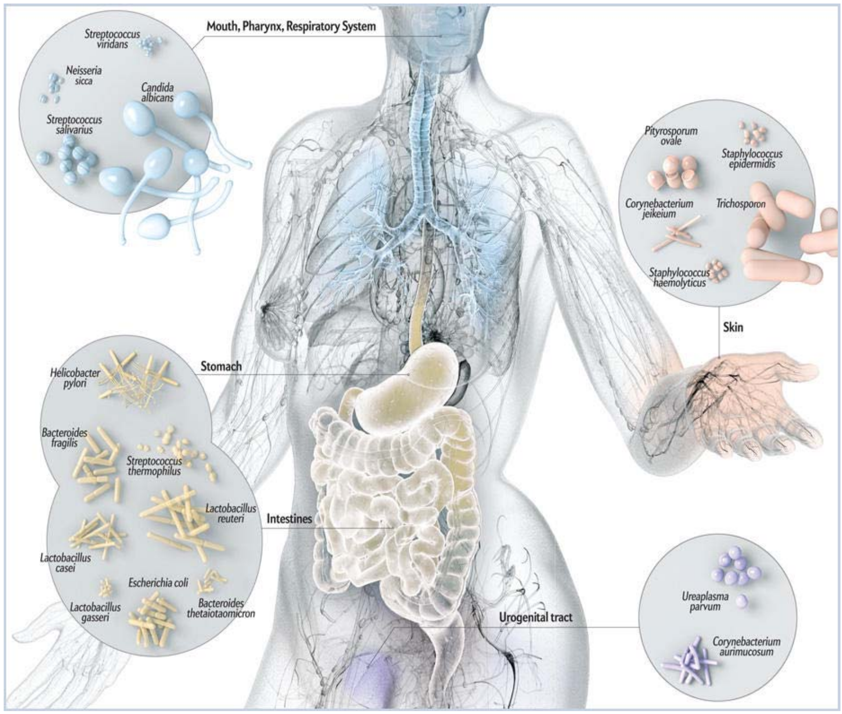
probiotic strains
…
Catalase
SOD
Reg3-a
…
Polyphenols & aromatics
…
Vitamin C

inflammation

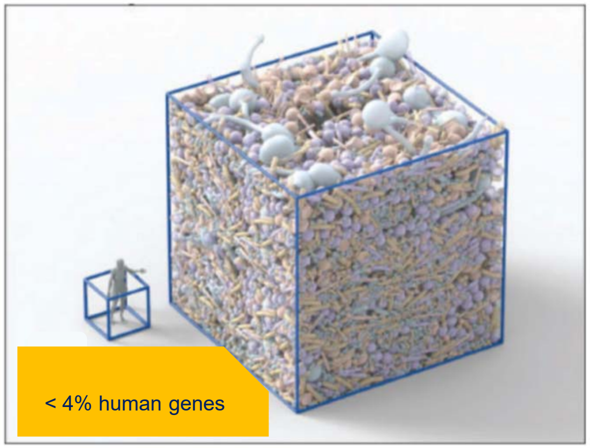
Commensal strains, probiotic strains, small molecules

The human is microbial, ecosystem and symbiosis

100 000 000 000 000



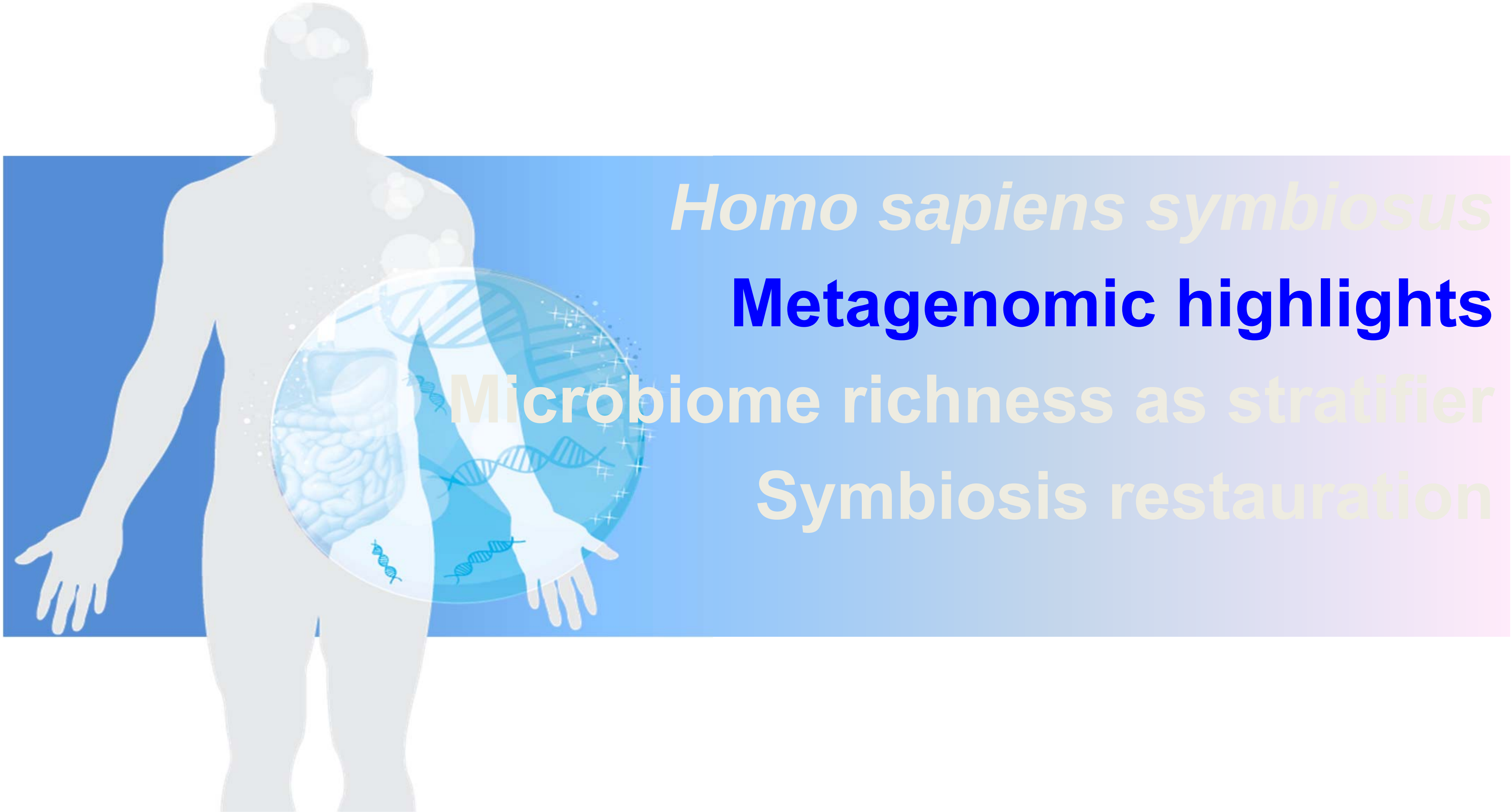
23,000 Human genes	600,000 microbial genes*



science of the microbiome
is changing the landscape

** per individual*

Grice et al, Annu Rev Genomics Hum Genet 2012
HMP Consortium et al., Nature 2012



Homo sapiens symbiosus

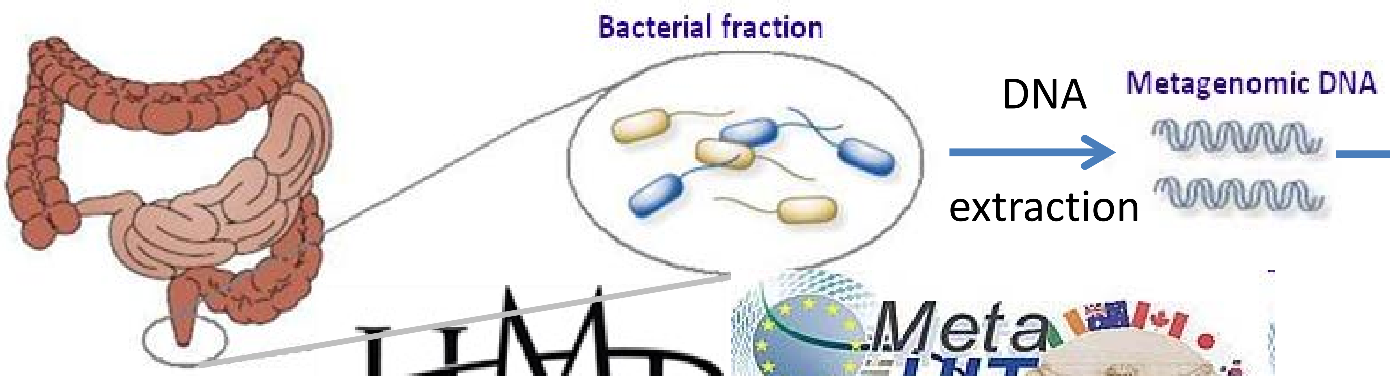
Metagenomic highlights

Microbiome richness as stratifier

Symbiosis restauration

Metagenome: combined genomes of all dominant microbes

A technical revolution of the 21^{rst} century



DNA extraction → Metagenomic DNA → **Whole Genome Shotgun sequencing**

↓

Assembly and annotation

↓

HMP

MetaHIT

IHMC

Lessons from early human intestinal tract metagenomics:

Reference gene catalogs, highlighting both core metagenome & rare genes Qin ***Nature 2010*** ;
Li ***Nature Biotech 2014***

Co-abundant gene clustering and metagenomic species Nielsen ***Nature Biotech 2014***

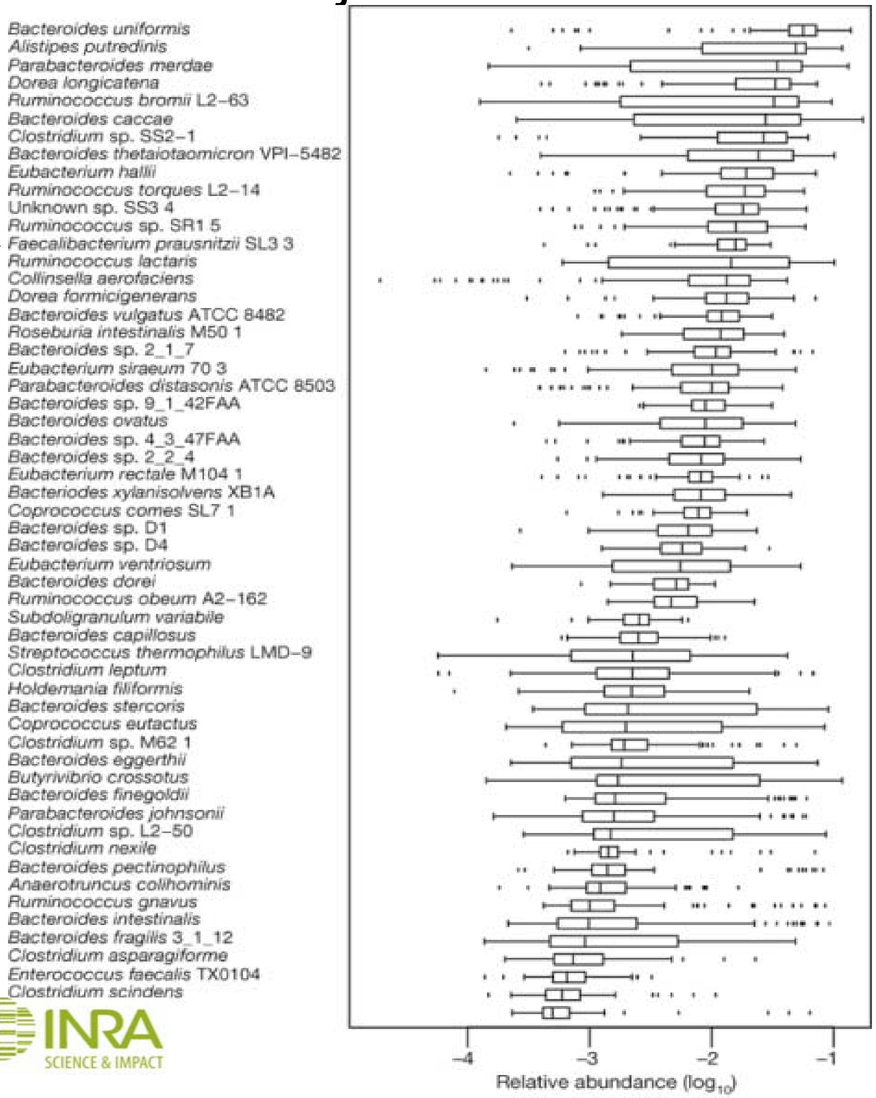
Preferred ecological arrangement as stratifier : 3 enterotypes Arumugam ***Nature 2011***

Low gene count as stratifier Cotillard ***Nature 2013***

Microbiome composition as stratifier : diagnostic signatures & predictive models..
in T2D Qin ***Nature 2012***, **in Obesity** Le Chatelier ***Nature 2013***, in Liver cirrhosis Qin ***Nature 2014***

A shared phylogenetic / metagenomic core

57 species present in 90% of subjects



18 species present in ~100% of subjects

→ *Faecalibacterium prausnitzii SL3 3*

Roseburia intestinalis M50 1

Bacteroides vulgatus ATCC 8482

Bacteroides sp. 9_1_42FAA

Ruminococcus sp SR1 5

Coprococcus comes SL7 1

Bacteroides sp. 2_1_7

Bacteriodes xylanisolvens XB1A

Ruminococcus torques L2-14

Bacteroides sp. 2_2_4

Bacteroides sp. D4

Bacteroides dorei

Ruminococcus obeum A2-162

Ruminococcus lactaris

Bacteroides capillosus

Bacteroides finegoldii

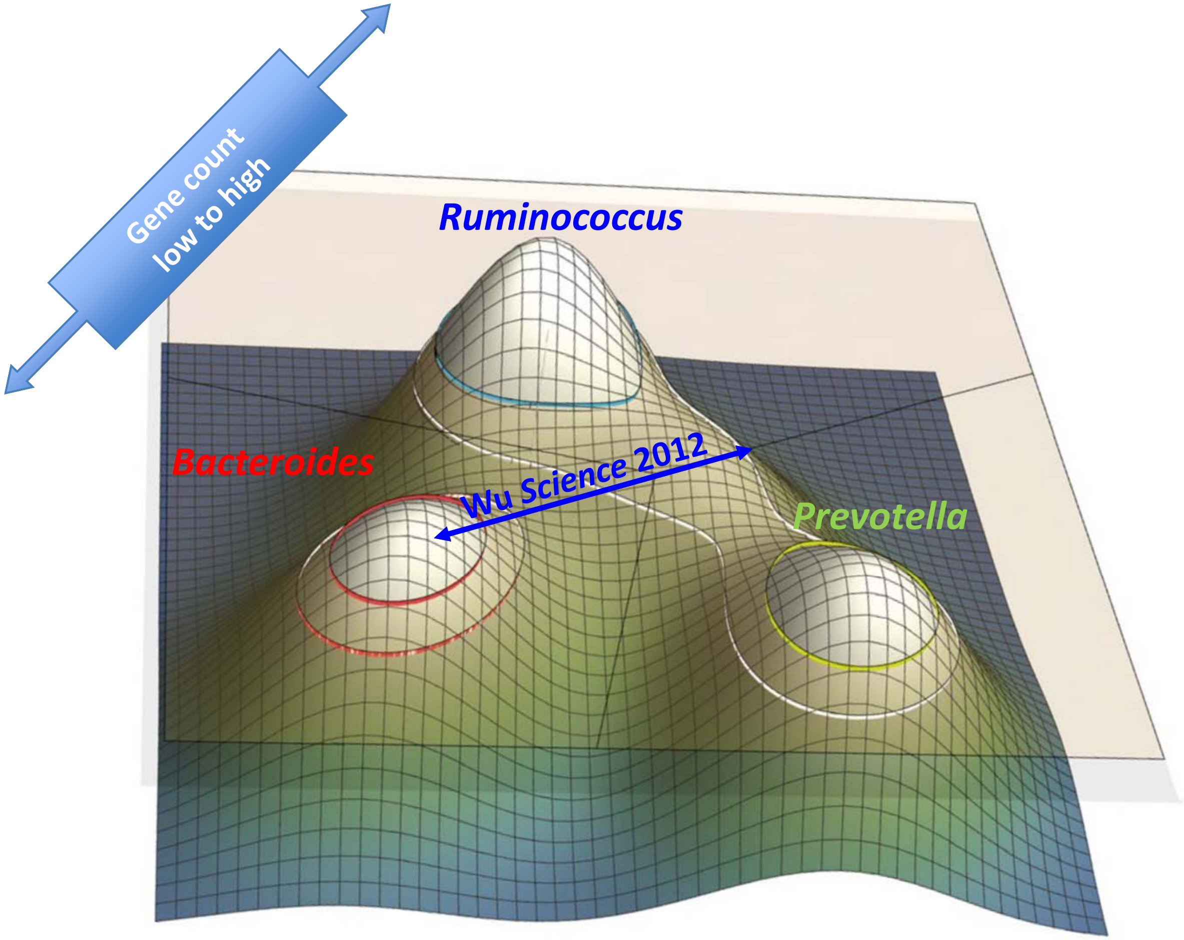
Clostridium sp M62 1

Clostridium nexile

Out of ~200 dominant species per individual

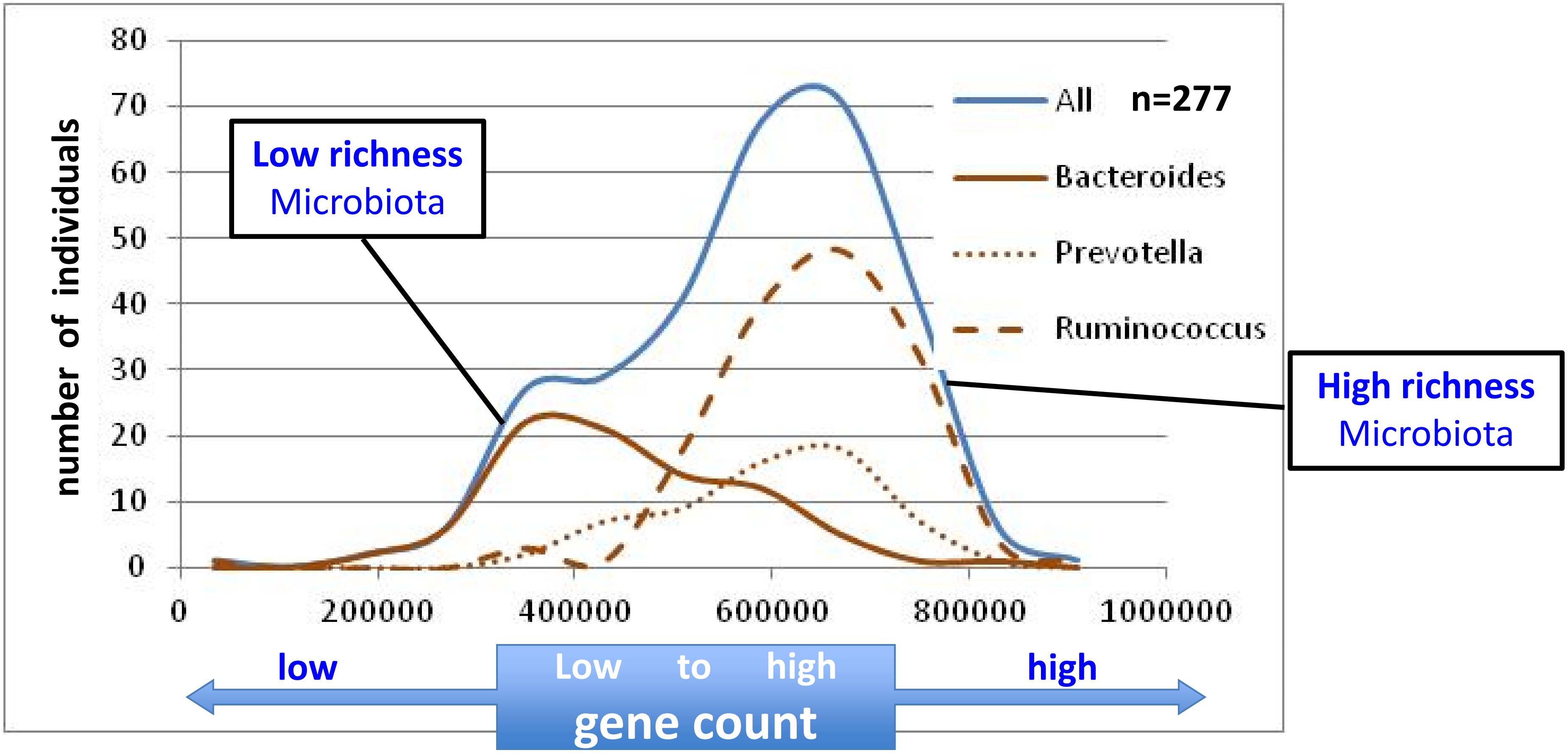
Qin et al. Nature 2010

Human microbiomes differ at the level of ecological arrangements with 3 preferred patterns: the enterotypes



De Arumugam *Nature* 2011 to Costea *Nature Microbiol* 2018

Human microbiomes differ at the level of gene richness (diversity)

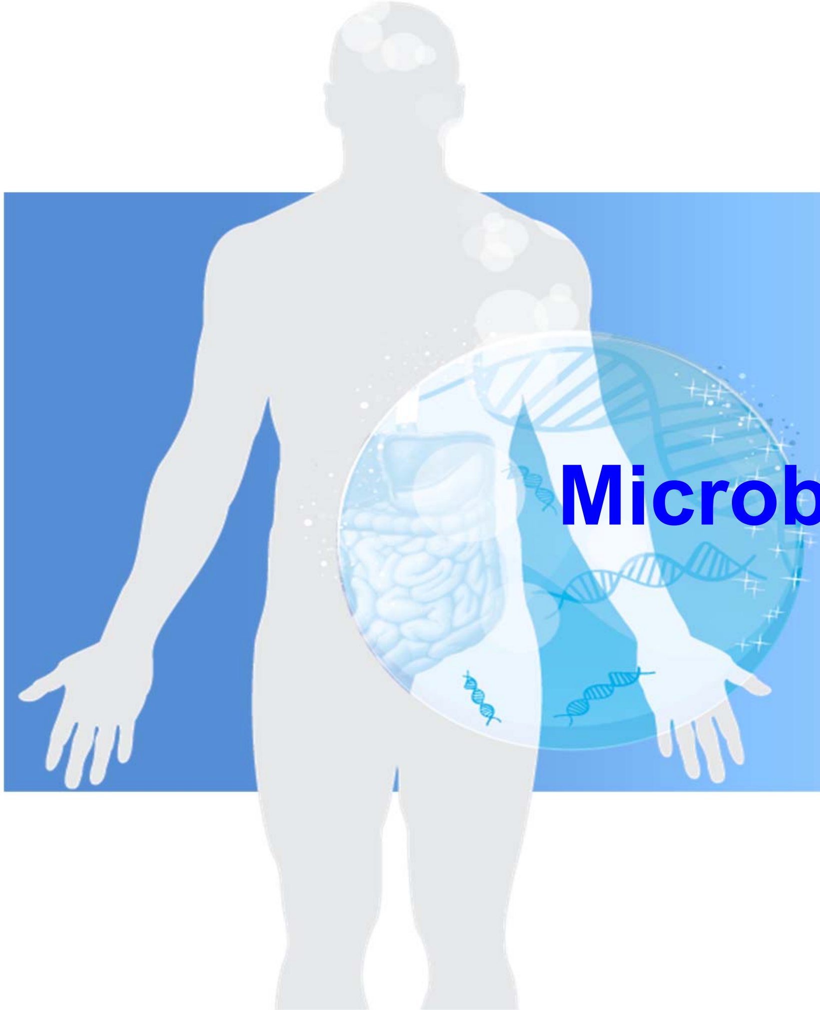


68 « species » significantly linked to gene count (richness/diversity)

Low gene richness is a key stratifier in chronic conditions, associated with worst phenotypes, non response to treatments and high risk of comorbidities

Metagenomic view of the fecal microbiota

- Each dominant microbiota gathers on average over 600,000 genes representing 100's of bacterial species (Qin et al Nature 2010) ; this is over 25 fold the size of the human genome for each individual.
- A reference catalog of some 10 million genes.
- A fairly large proportion of genes constitute a metagenomic core.
- Individuals are nonetheless different by genes, gene richness, metagenomic species and enterotypes (Arumugam et al. Nature 2011)
- The microbiota can be characterized by quantitative metagenomic profiling. (Cotillard et al. Nature 2013)
- Genomes of yet uncultured metagenomic species can be assembled (Nielsen et al. Nature Biotech 2014)



Homo sapiens symbiosus

Metagenomic highlights

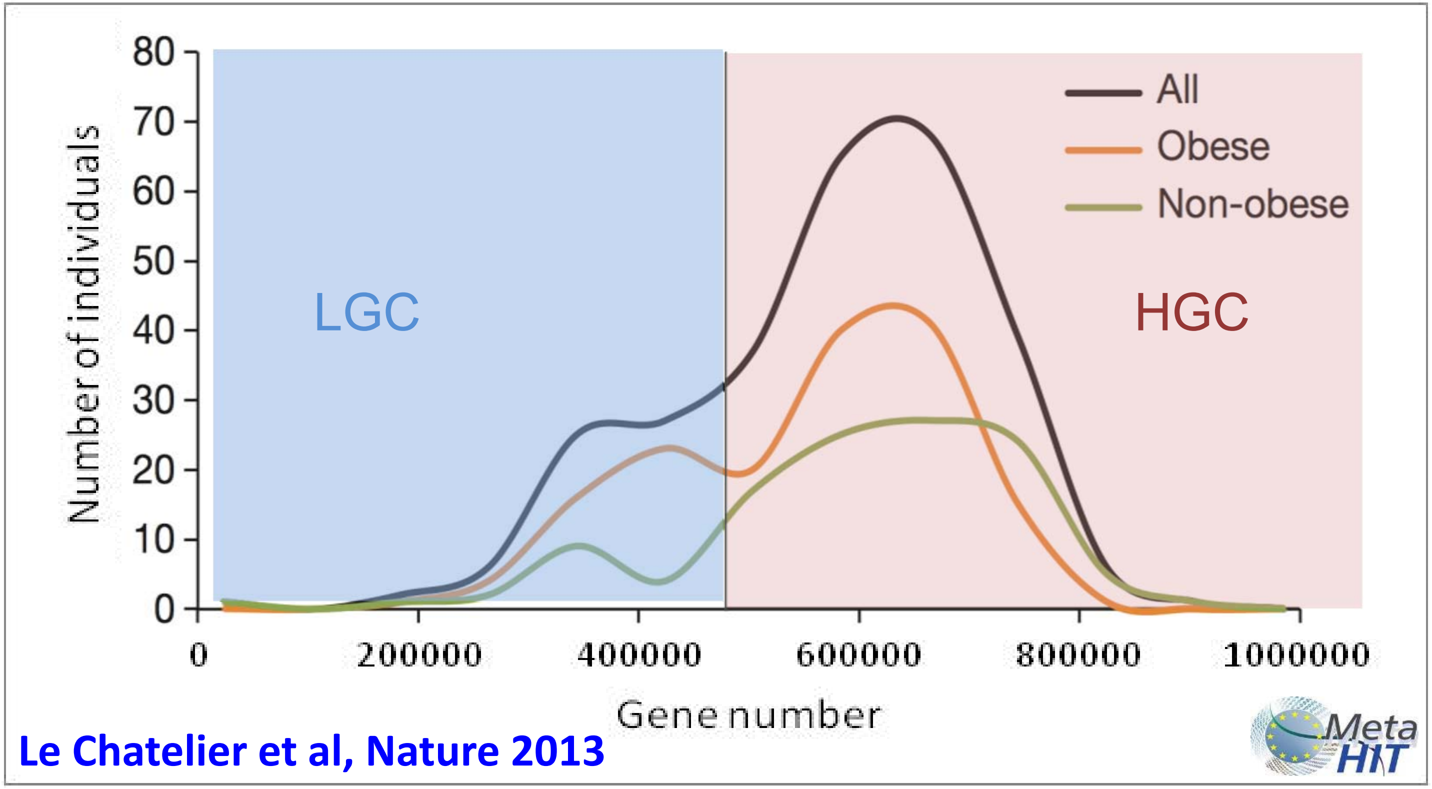
Microbiome richness as stratifier

Symbiosis restauration

Microbiome gene count as stratifier

Microbiota gene count / diversity is a health-associated stratifier

Low to High
gene count (French or Danes)

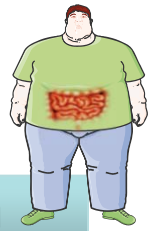


Le Chatelier et al, Nature 2013

Low gene count (**low bacterial richness**) individuals have less healthy metabolic & inflammatory traits: increased adiposity, dyslipidaemia, inflammation, insulin resistance, that predispose to type 2 diabetes, cardio-vascular disease, …

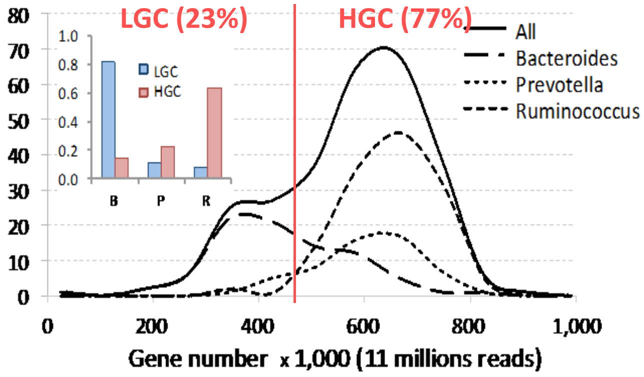
Microbiota, obesity and metabolisme :

Low gene count (LGC) is a signature of risk



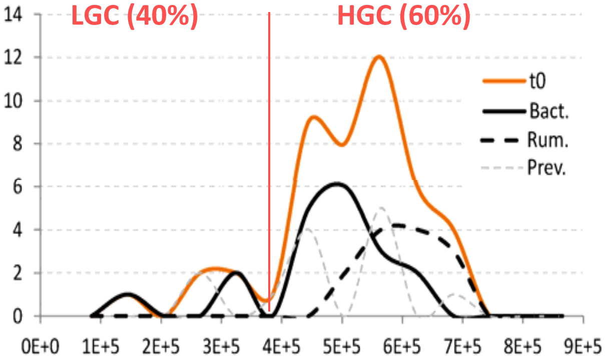
METAHIT

292 subjects (lean & obese)



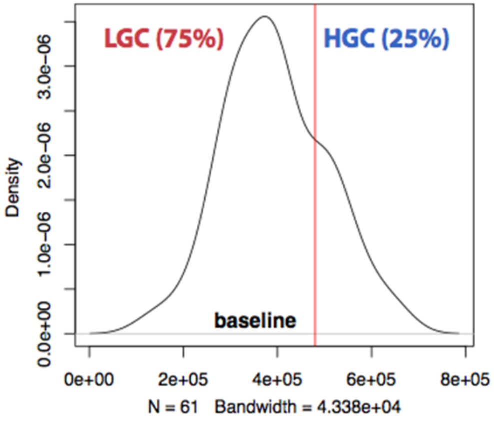
Micro-Obese

49 subjects (overweight & obese)



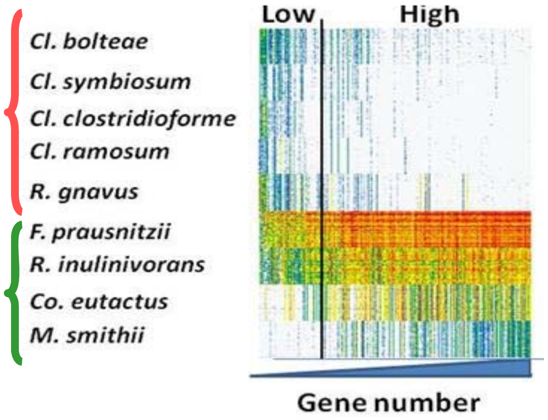
MicroBaria

61 sujets (bariatric candidates)



low gene count (LGC) = less healthy

- Increased adiposity
- Insulino-resistance
- Increased dyslipidemia +++
- Inflammatory phenitype (systemic and adipose tissue)



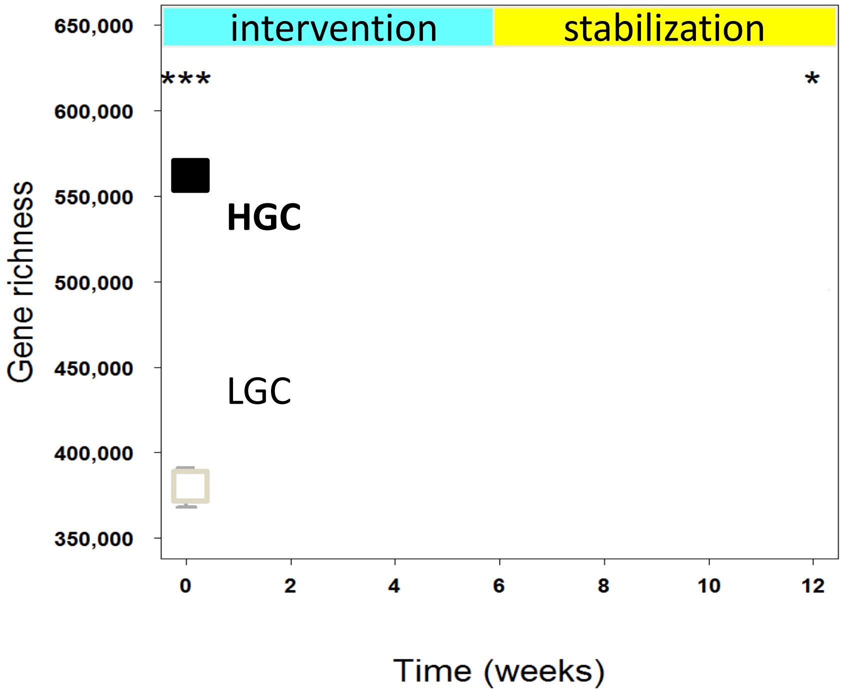
↗ Pro-inflammatory

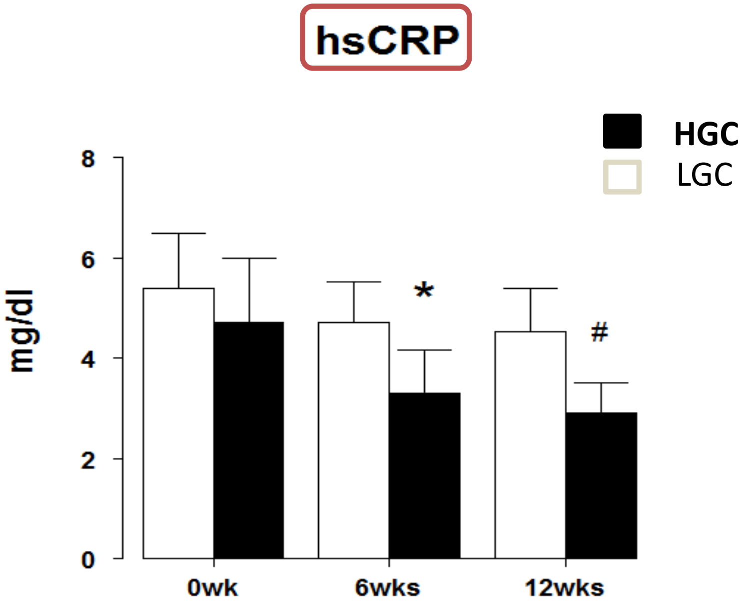
including all *Bacteroides sp*

↘ Anti-inflammatory

Le chatelier et al Nature 2013; Cotillard et al Nature 2013; Aron J, Prifti E, Clement K unpublished

Low richness microbiota in obesity predicts a lesser response to a caloric restriction with a low fat, high protein and high diverse fiber diet





Patients with low gene count microbiota do not respond as well to the dietary intervention (weight loss, TG, Disse index, hsCRP ,..)

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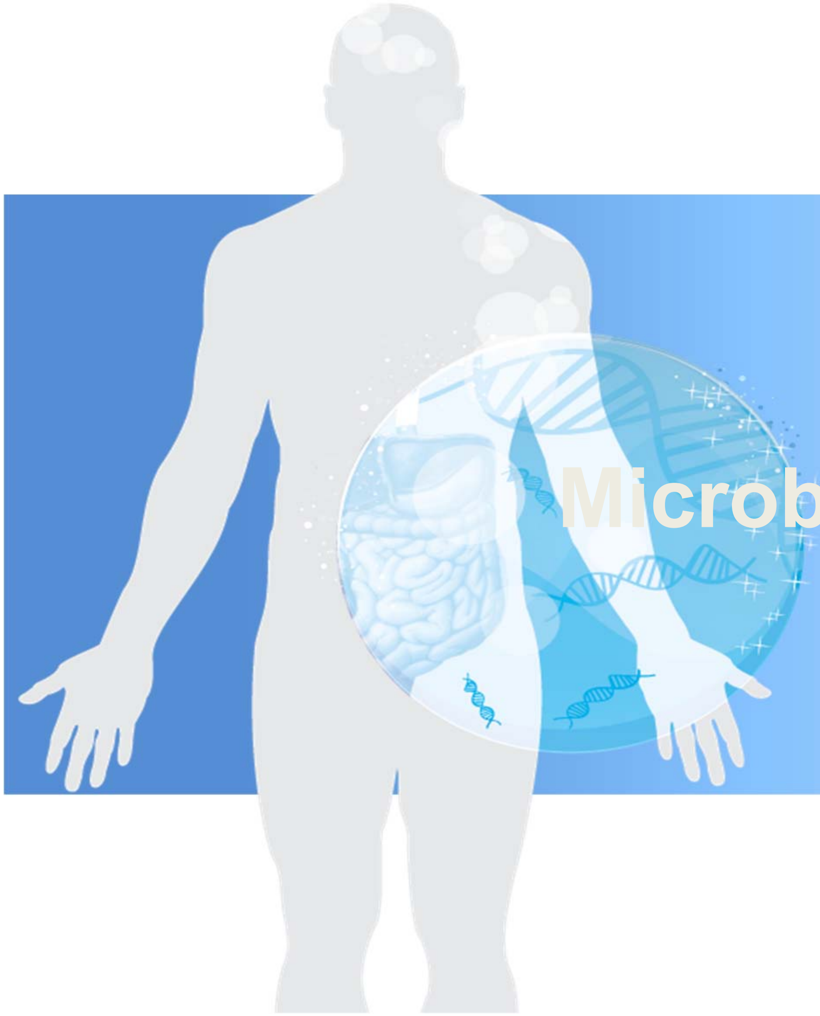

Homo sapiens symbiosus

Metagenomic highlights

Microbiome richness as stratifier

Symbiosis restauration

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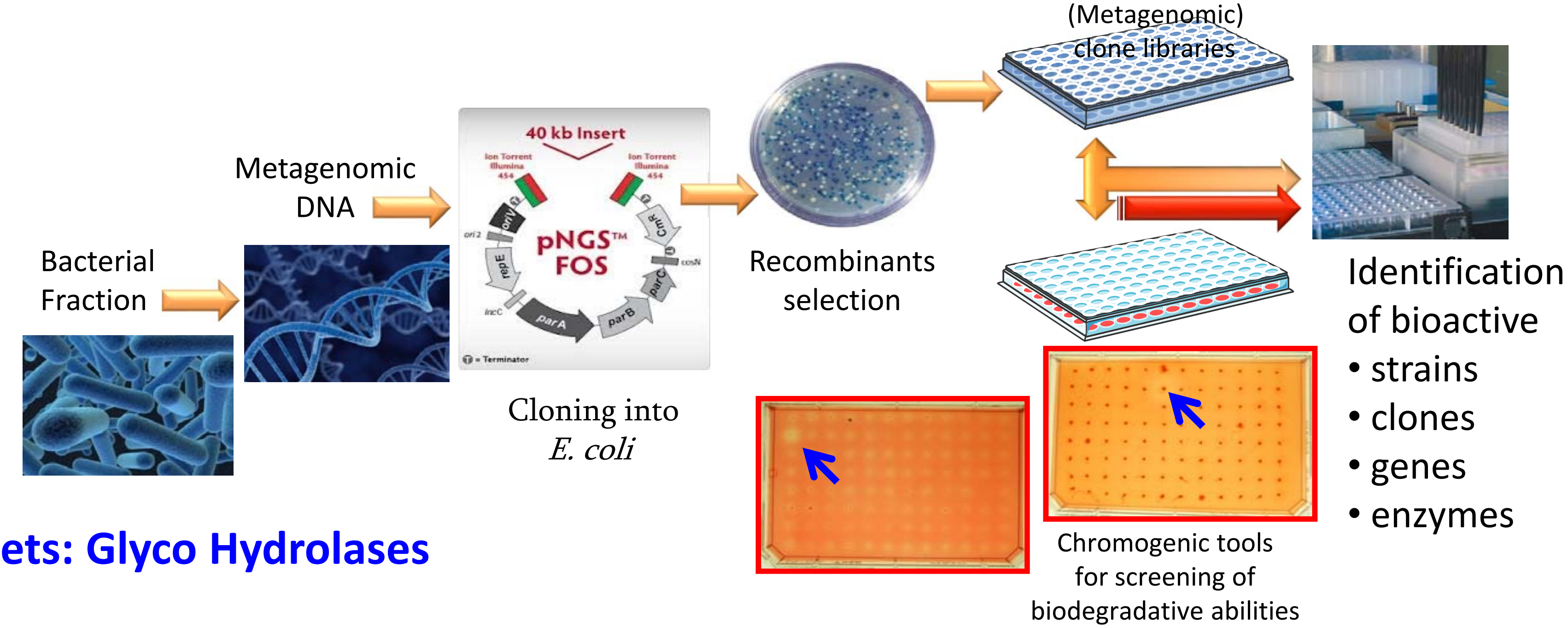
Functional metagenomics exploration of fiber degradation

Sampling & DNA extraction → Cloning, recombinants selection → Homogenous DNA library → Screening

Exploring food-microbe interface and its components



Bacterial Fraction

Metagenomic DNA

Cloning into *E. coli*

Recombinants selection

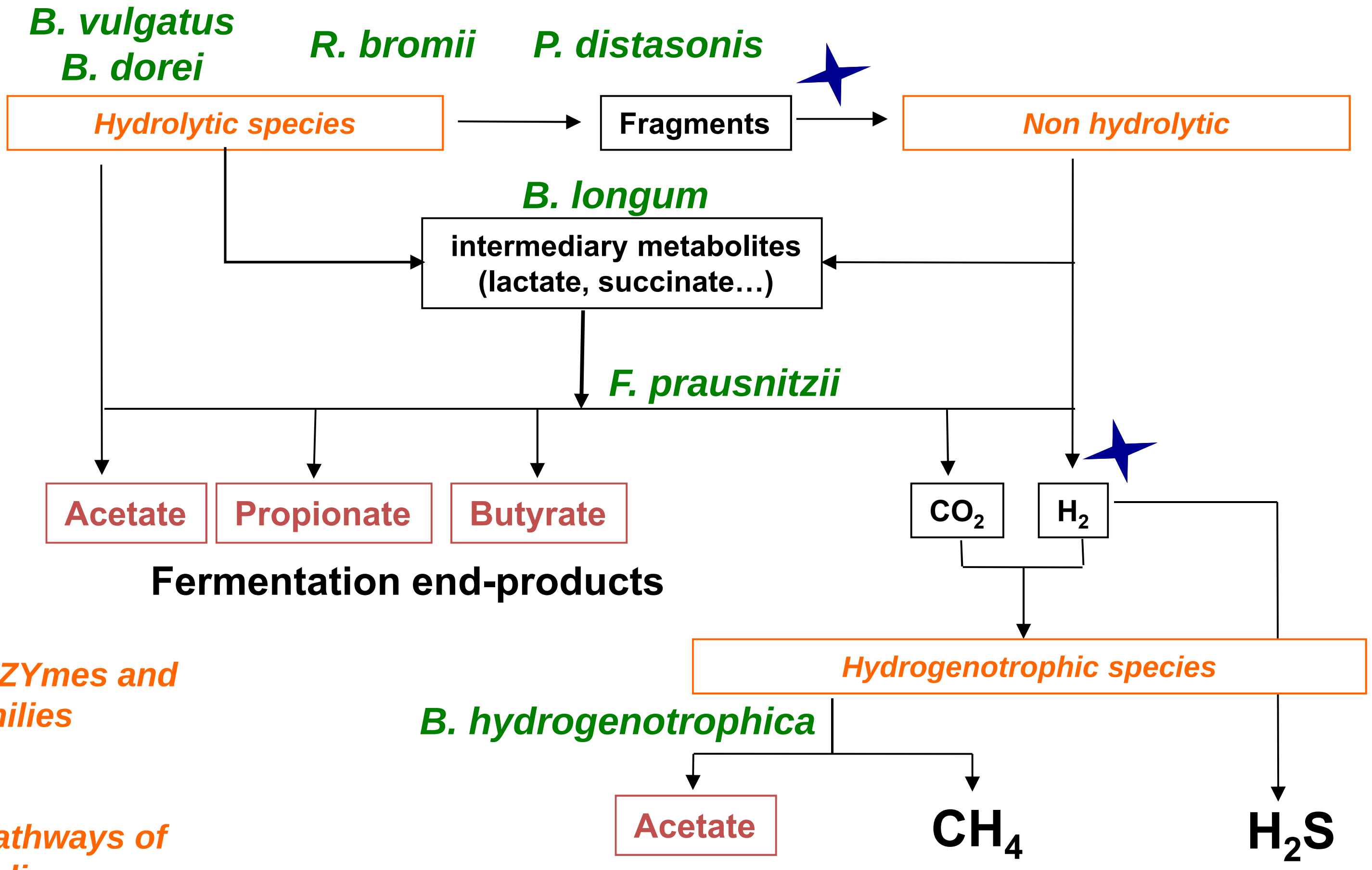
(Metagenomic) clone libraries

Chromogenic tools for screening of biodegradative abilities

Identification of bioactive
- strains
- clones
- genes
- enzymes

Targets: Glyco Hydrolases

Fibers and the microbial ecosystem ; toward modeling and innovation



B. vulgatus
B. dorei

R. bromii

P. distasonis

Hydrolytic species

Fragments

Non hydrolytic

B. longum

intermediary metabolites
(lactate, succinate…)

F. prausnitzii

Acetate

Propionate

Butyrate

CO_2

H_2

Fermentation end-products

Hydrogenotrophic species

B. hydrogenotrophica

Acetate

CH_4

H_2S

Tasse et al,
Genome Res 2010

156,000 clones métagénomiques

- *310 actives ; 73 CAZYmes and 9 novel GH families*

Ceccini et al, 2012

- *102 genes & 3 pathways of prebiotic metabolism*

Patrascu et al, Sci Reports 2017

- *3 novel glycohydrolases*

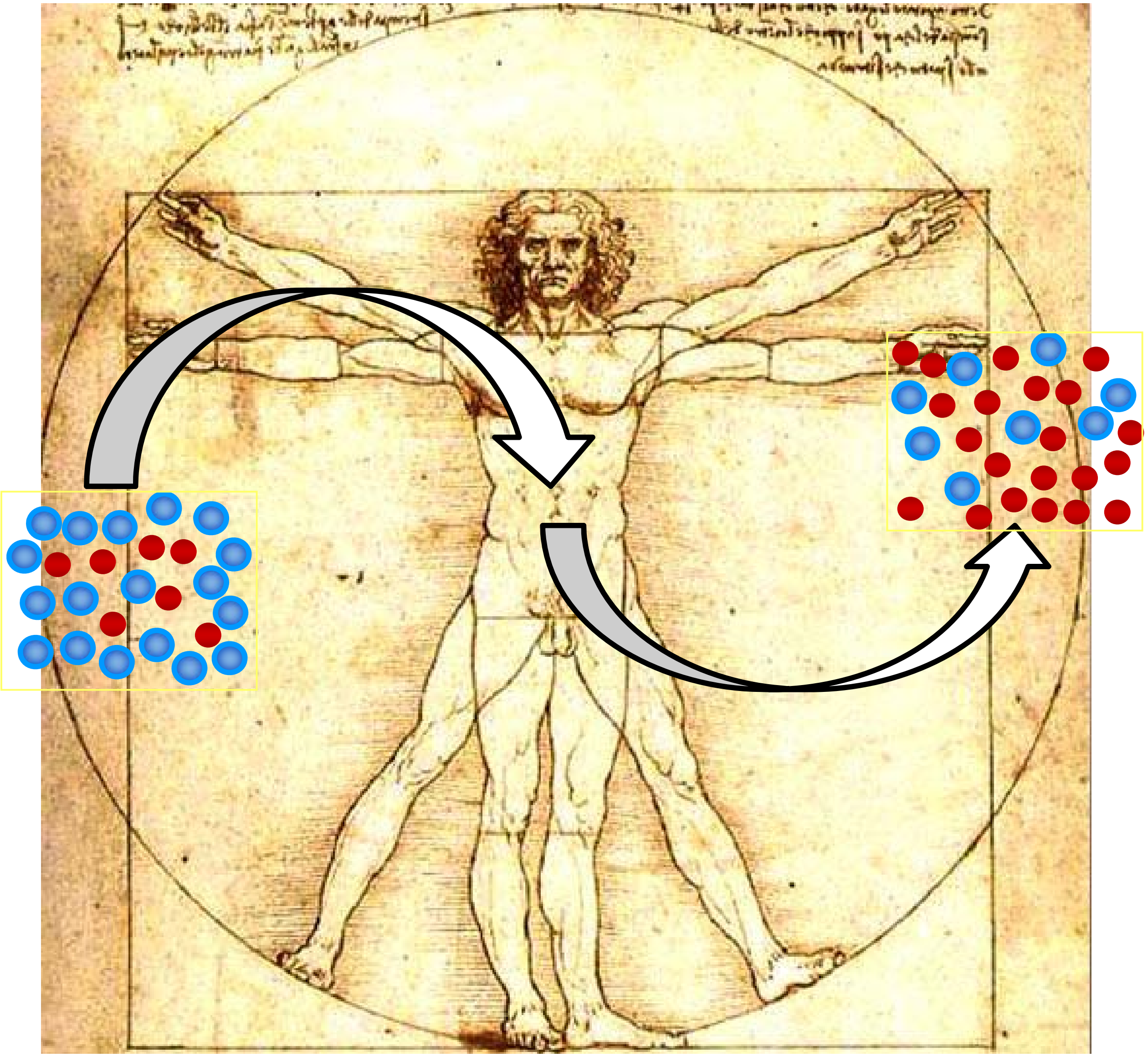
Live Biotherapeutic Products:

Bioactive commensals as new-generation probiotics

Bacteroides fragilis	PSA	Mazmanian	-
Faecalibacterium prausnitzii	MAM?	Sokol, Langella	Nextbiotix
Eubacterium hallii	?	Nieuwdorp	Caelus
Akkermansia muciniphila	Amuc_1100	Cani, de Vos	A-mansia
Roseburia intestinalis	flagellin	Kelly	4D Pharma
Blautia hydrogenotrophica	metabolism	Bernallier	" "
Hafnia alveï	clpb	Fetissov	Targedys
Mixed spore formers	?	Honda	Vedanta
Segmented filamentous bacteria	?	Cerf-Bensussan	-
Christensenella spp	?	Ley	-

..oncobiotics..

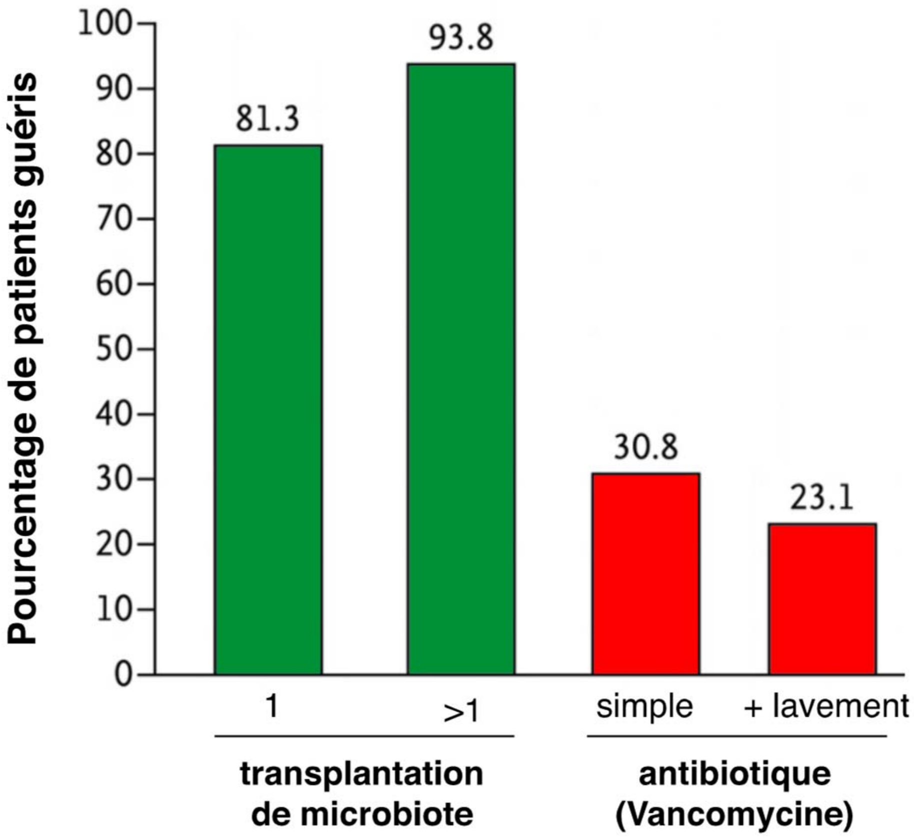
Fecal transplantation



Healthy microbiota

Disease-associated microbiota

Fecal microbiota transfer, acknowledged as a treatment in a single indication : recurrent *Clostridium difficile* infection



Clinical trial was interrupted at intermediary analysis :

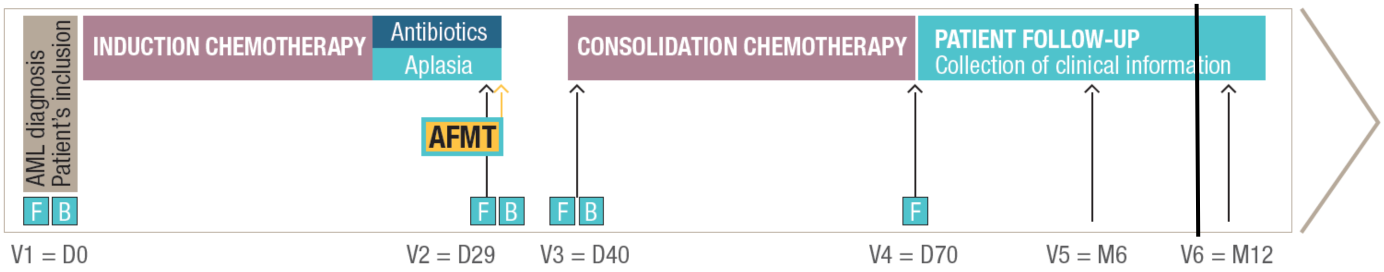
➔ non-ethical to go further owing to extremely high efficacy of the novel treatmentcompared to standard of care

➔ Acknowledged by regulator and applied daily to cure thousands of patients every year

Van Nood et al. *NEJM* 2013

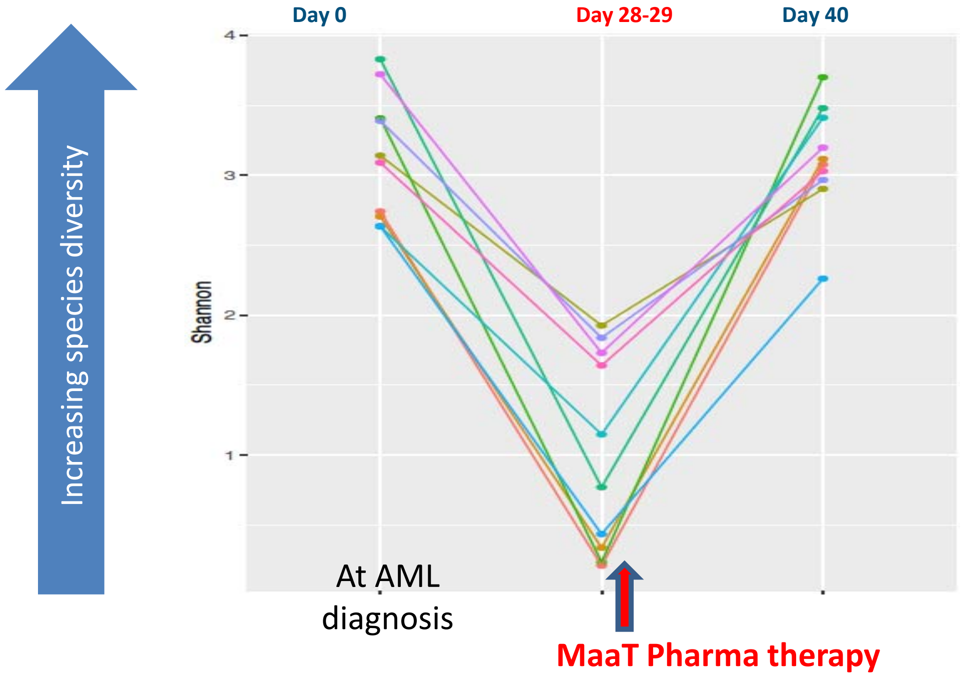
OdysséE study : autologous fecal microbiota transfer to restore symbiosis post-treatment in acute myeloid leukemia

By MaaT Pharma



June 2018
(final assessment)

F: Faeces collection - B: Blood collection



Autologous **diversity restoration** in AML patients :

- **Species level** (on average **> 90%** of richness, Shannon diversity index and Simpson diversity index restoration)
- **Gene level** (on average **>80%** of richness, > 90% for Shannon diversity index and Simson diversity index restoration)

25 AML patients treated ;
20 per protocol ;
10 fully analyzed (adjacent figure)

www.MaaTPharma.com

Take home messages :

- **Humans share a core microbiome and yet they differ** by genes, species, enterotypes (ecology) and gene count (microbiota diversity).
- **microbiome gene count is a key stratifier** in several immune disorders including major diseases of modern world, that have increased in incidence since 1950’s
- **dysbiosis is an altered state of microbe-host symbiosis**, with auto-aggravating signals from both sides.
- **new paradigm : multi-target modulation should be considered** for personalized nutrition, prevention and therapy
- **micronutrients, fibers and live microbes** may be strategic bioactives for the maintenance, preservation or restoration of man-microbe symbiosis

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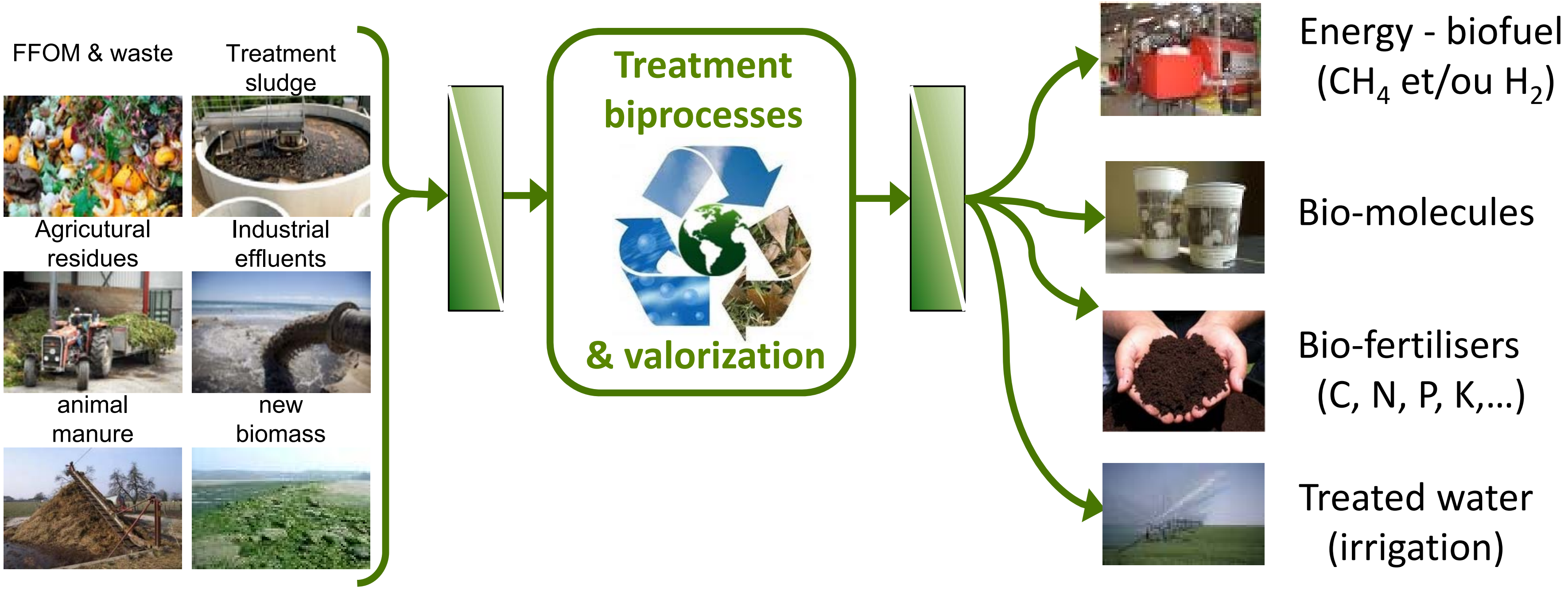
Ideally equipped for structural and functional assessment of the microbiota

Laboratoire de Biotechnologie de l'Environnement
INRA Environmental Biotech Lab, Narbonne
Environnemental Bioraffinery

- ✓ Address uses and needs
- ✓ Make high value of residues
- ✓ Think circular economy and sustainability



FFOM & waste

Treatment sludge

Agricutural residues

Industrial effluents

animal manure

new biomass

Treatment biprocesses & valorization

Energy - biofuel (CH_4 et/ou H_2)

Bio-molecules

Bio-fertilisers (C, N, P, K,…)

Treated water (irrigation)

*FInE/**Blottière** lab,*
Micalis Institute

Joël Doré,
Nicolas Lapaque,
Catherine Juste,
Christel Maillet-Béra
Jean-Marc Lelièvre
Alexandre Jamet
Maarten van de Guchte
et al....

MetagenoPolis

S. Dusko Ehrlich
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Florence Haimet
Nicolas Pons
Emmanuelle Le Chatelier
Véronique Lejard
Florence Levenez
Jean-Michel Batto
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Sven Pettersson et col. (Karolinska Institute)
Maria Rescigno (IEO, Milan)
Oluf Pederson (Novo Nordisk Fundation, Copenhague)
Francisco Guarner (Val Hebron Hosp., Barcelone)
Mark Morisson (U of Queensland, Brisbane)

http://gutmicrobiotaforhealth.com/

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Merci
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ChenHong Zhang
Julien Tap
Stanislas Mondot
Omar Lakhdari

Philippe Langella
and col.

Bruno Pot
Corinne grangette
and col.

European Community
& ANR-France

S Dusko Ehrlich, Jean Weissenbach (Genoscope, Evry), **Wang Jun** (BGI, Shenzhen), **Peer Bork** (EMBL Heidelberg)**, Francisco Guarner** (Val d'Hebron Hospital Barcelona)**, Oluf Pedersen** (SDC Copenhagen)**, Maria Rescigno** (IEO Milan)**, Liping Zhao** (Shanghai JiaoTong University), **Jim Versalovic** (Baylor College of Medicine, Houston), **Baghi Singh** (Western Ontario, London) and **EU-MetaHIT and IHMS Consortia**

Karine Clément (INSERM U972, CR des Cordeliers), **Denis Le Paslier & Eric Pelletier**, (CEA-Genoscope), **Liping Zhao** (Shanghai JiaoTong University) and **ANR MicroObese consortium**

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PLATFORM OF EXCELLENCE DEDICATED TO QUANTITATIVE AND FUNCTIONAL METAGENOMICS, FUNDED BY FRENCH GOVERNMENT'S *FUTURES INVESTMENTS*

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Meta HIT

Cross Talk

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Microbiota as a therapy

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