Standards for Microbiome studies - getting it right from the start -

Joël Doré, INRA, France

Joël Doré - disclaimer

- BMS
- Biocodex
- Danone
- Jansen
- Enterome
- MaaT Pharma
- Indigo Therapeutics

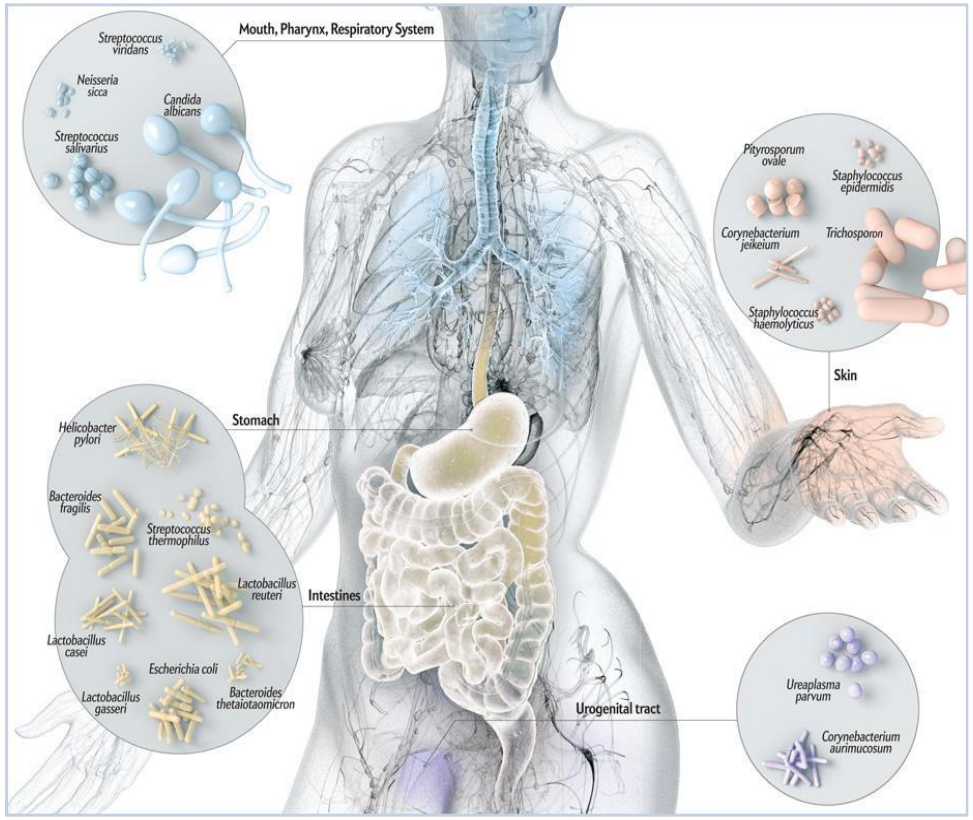
Outline of presentation:

- **Why perform microbiome studies in the clinical setting ?**
  - For a medicine of human-microbes symbiosis
  - Critical transitions rather than a health-disease continuum
  - Interaction between microbiome and clinical management as opportunities

  a large spectrum of microbiome studies…
- How ? : need for standards and major pitfalls to avoid
  - Implement rigorous pipelines relying on standardized procedures
  - Be prepared for complexity w inter-individual variations
  - Anticipate confounders
- Resources you may use…

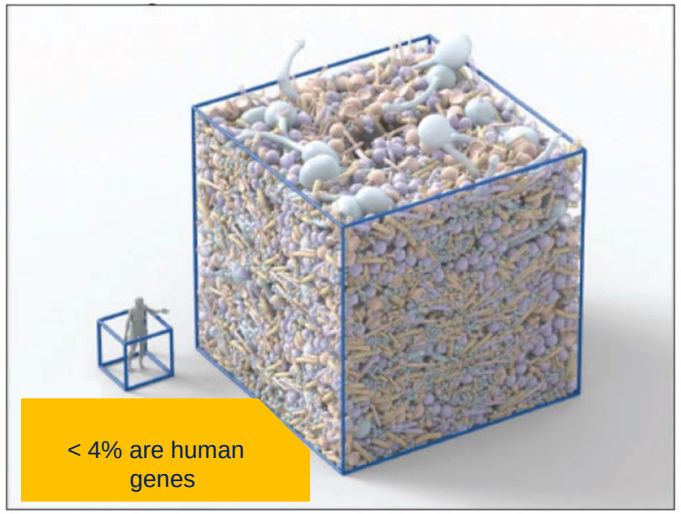
Humans are microbial

100 000 000 000 000



23,000
Human genes

600,000
microbial genes*

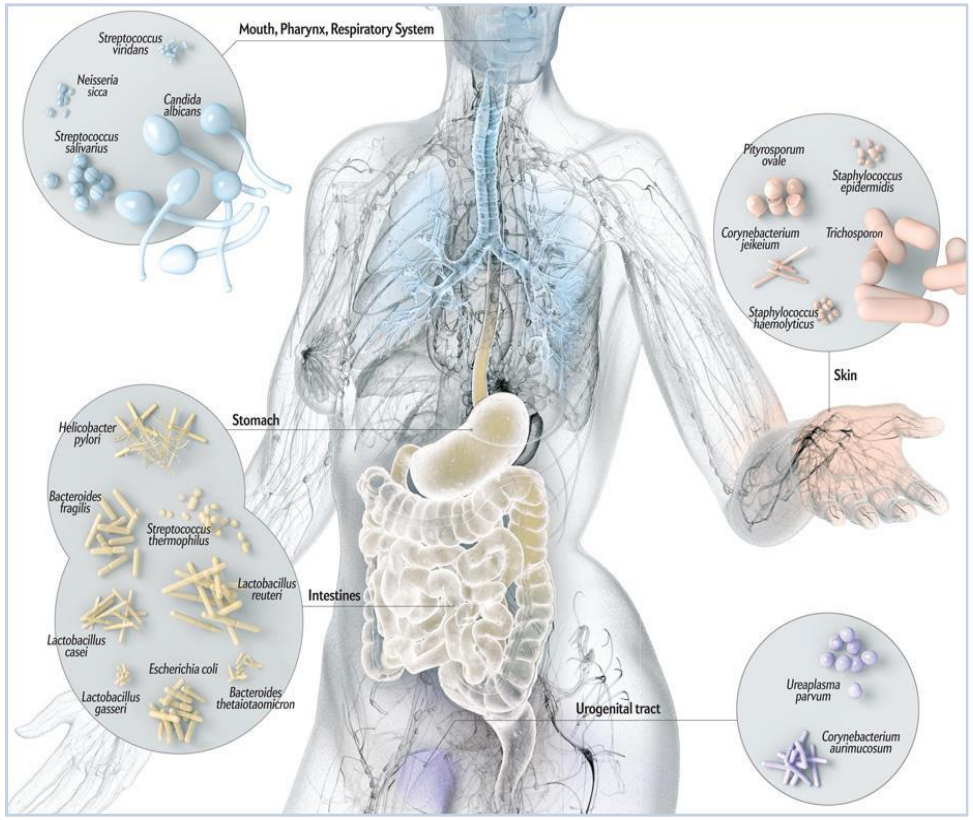


Microbiome science
is changing the landscape

** per individual*

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

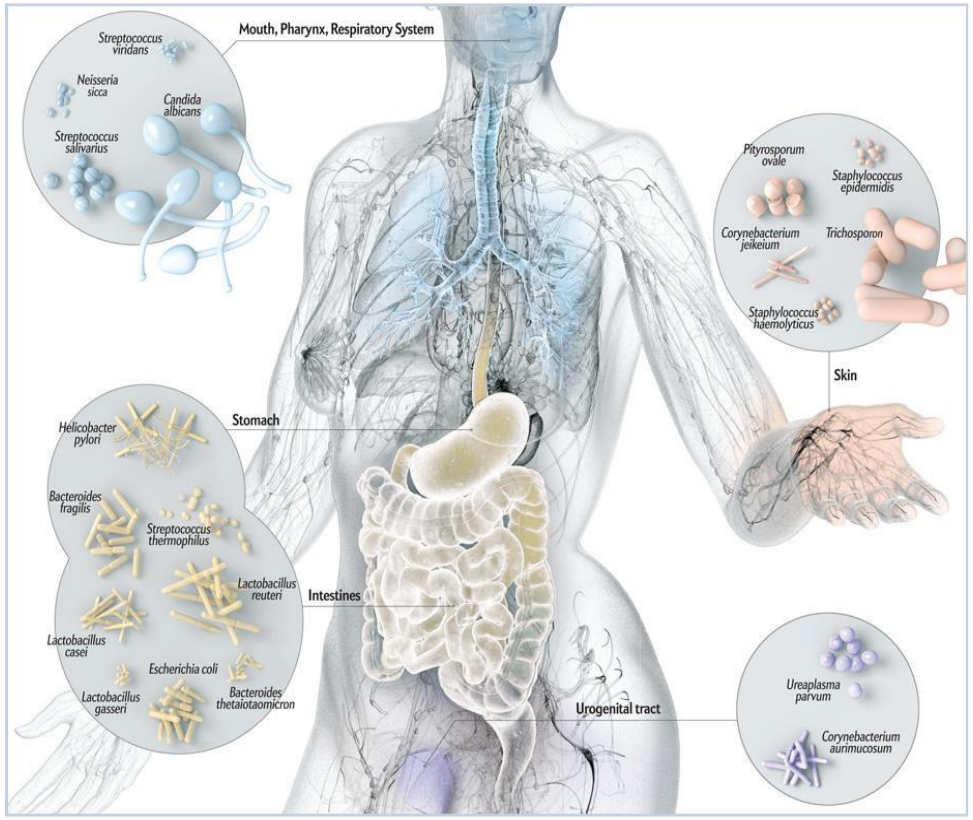
Microbiome, a key player: liability & asset



interacts with nutrition and with clinical management:

- Responds to diet
- Modulates bioavailability of actives
- Activates drugs
- Inactivates drugs (digoxin)
- Re-activates drugs in the gut (irinotecan)
- Acts as adjuvant (in cancer therapy)

Microbiome, a key player: as a tool ; as a drug …



Can serve monitoring and clinical purposes:

- Source of biomarkers ;
- Mediator of treatment efficacy ;
- Treatments (bugs as drugs)
- …

underpinning the large spectrum of microbiome studies

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  a large spectrum of microbiome studies…
- **How ? : need for standards and major pitfalls to avoid**
  - Implement rigorous pipelines relying on standardized procedures
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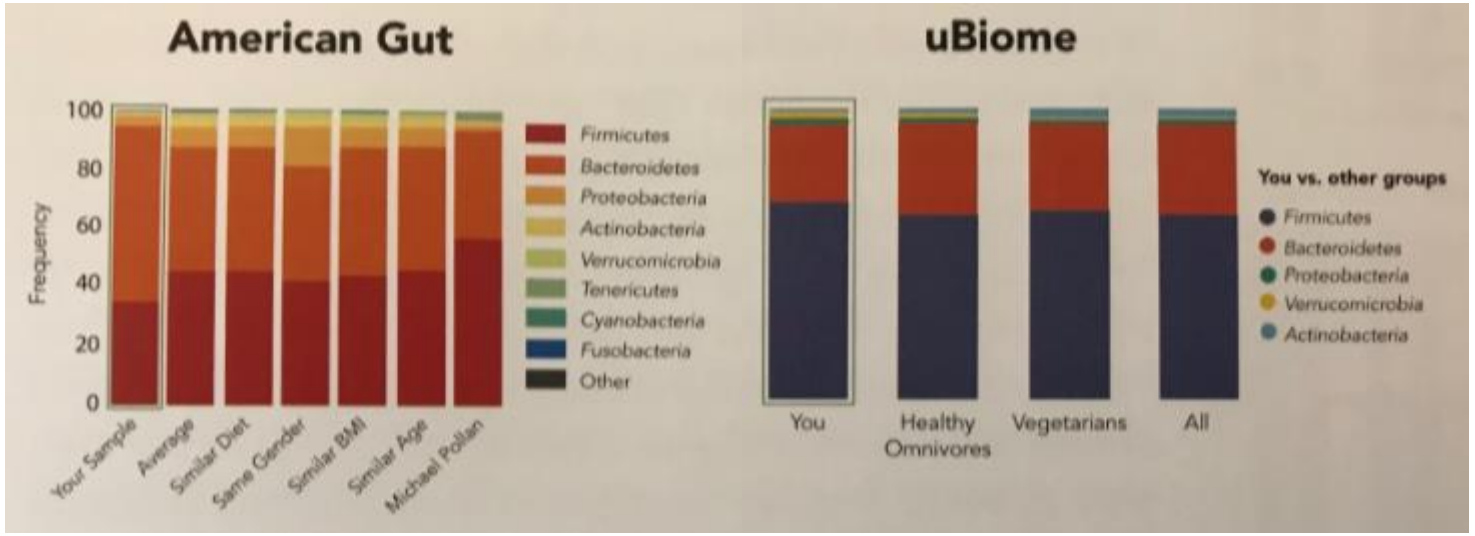
  - Anticipate confounders
- Resources you may use…

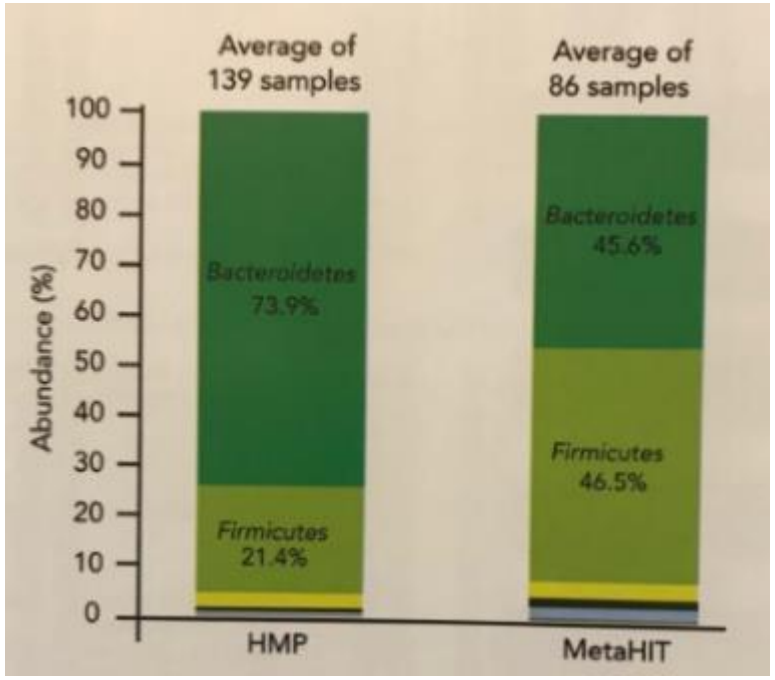
Rely on pipelines of standardized pre-analytical procedures …

so as not to see this, …, or this



Science News 2014

[same sample]



unpublished

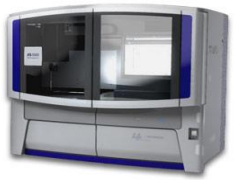
[different sample]

Quantitative metagenomics pipeline

Recueil d'échantillons → Séquençage → Construction de référence → Profilage des gènes → Bioinformatique & analyses statistiques

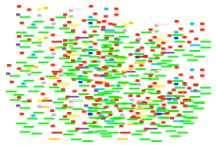
Stool sample

Total DNA



QC
Library preparation
SoLiD/Illumina
Sequencing

30-50 million sequences

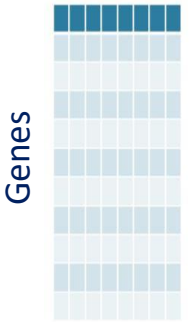


Known genomes

Reference Gene Catalog 10 Mio genes
Li NatBiotech 2014

Mapping to gene catalog

Gene counts

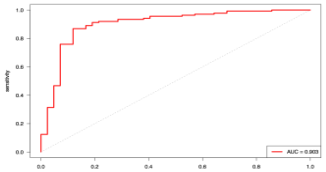


Individuals

Preprocessing / normalization and dimension reduction

relate to human data

Identify relevant microbial players
Nielsen NatBiotech 2014



Build and test prediction models

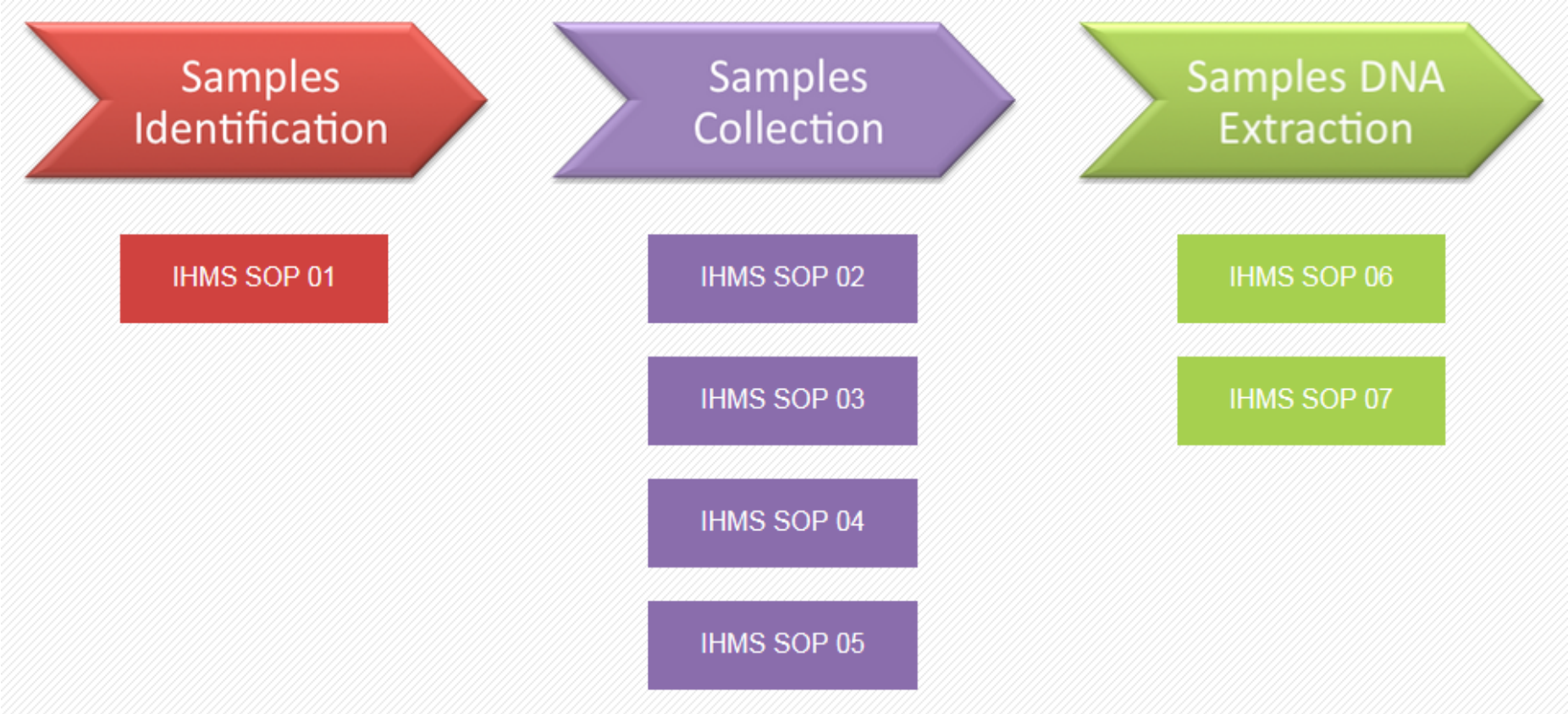
metagenopolis mgps.eu

Standardisation is crucial and every step matters, from bowel movement to data delivery

http://www.microbiome-standards.org

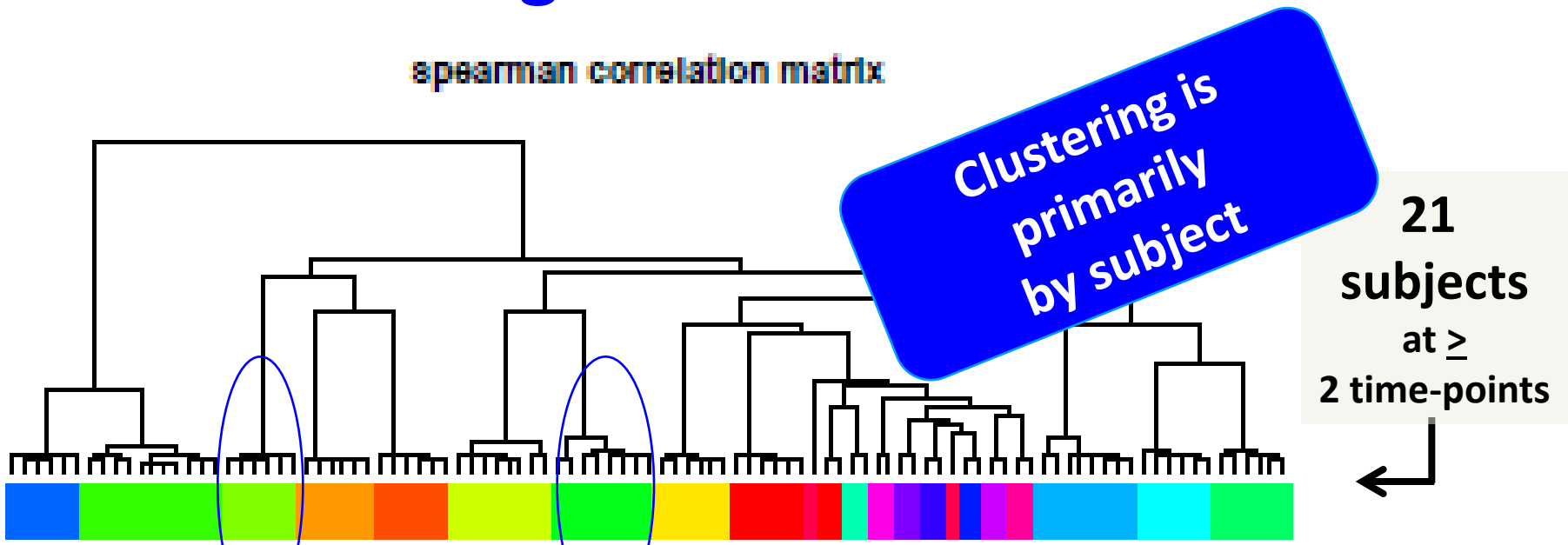
Rely on pipelines of standardized pre-analytical procedures

Collection and processing of human stool samples



http://www.microbiome-standards.org

Ecological resistance & resilience



Molecular scanning of microbiome followed by

- Computation of 2-by-2 similarity (spearman correlation)
- Cluster analysis dendrogram representation

Technical reproducibility > 98%

Similarity within subject over time > 92%

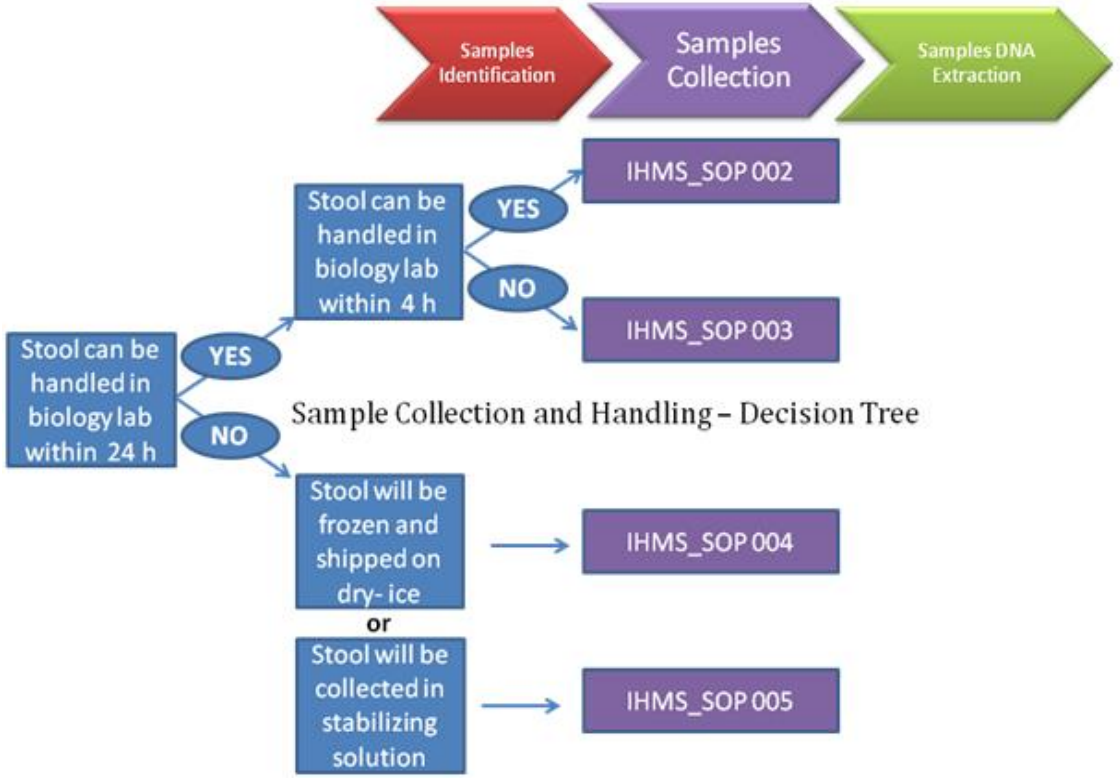
=> Benchmarking is possible ; comparing DNA QC and/or full microbiome profiles Resilience = recovery > 90% similarity within 2 months post antibiotics (de la Cochetiere 2005)

Sample collection sops

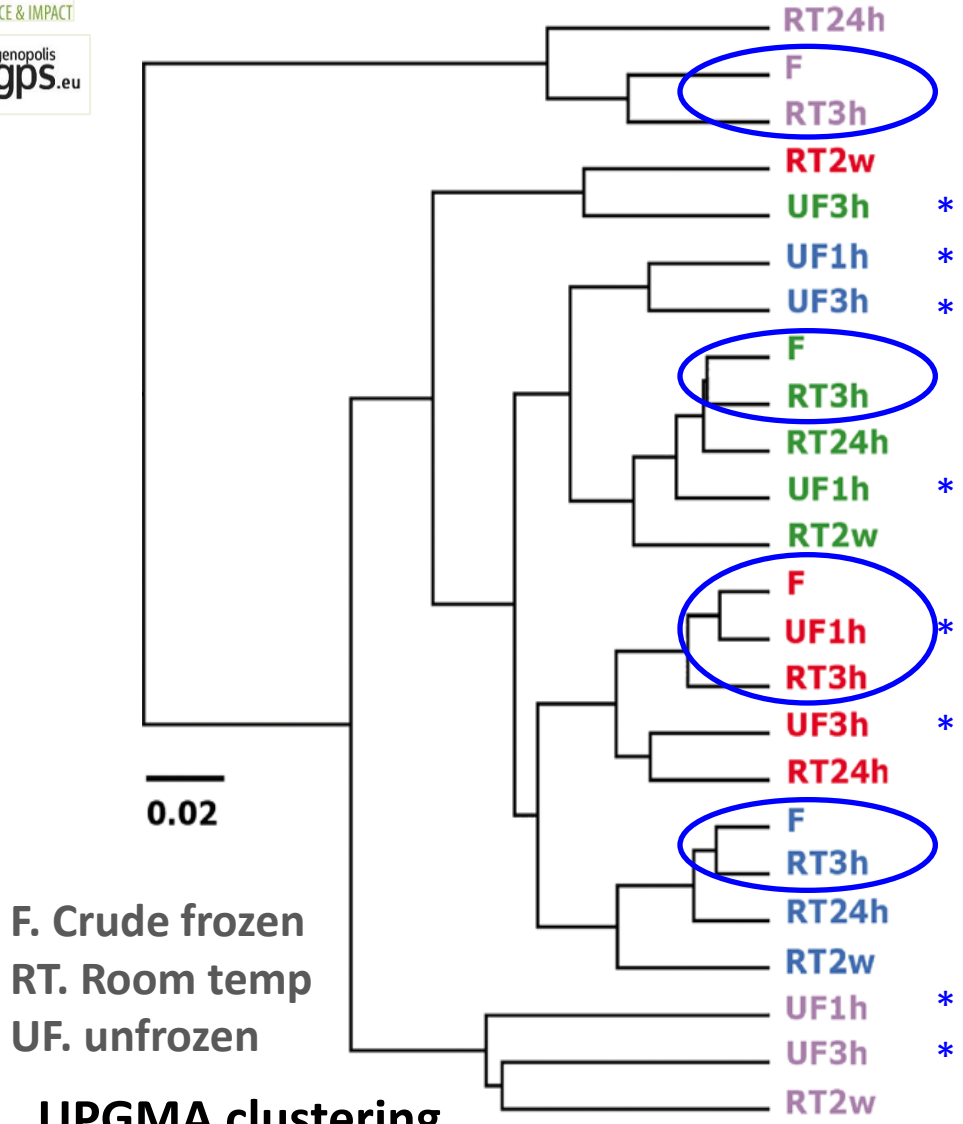
International Human Microbiome Standards

2. Collection and processing of human stool samples

Dore, J., Ehrlich, S.D., Levenez, F , et al. and IHMS Consortium (2015)



IHMS - WORKPACKAGE 2. STOOL COLLECTION



F. Crude frozen
RT. Room temp
UF. unfrozen

UPGMA clustering based on single gene profiling

Stool samples may be kept for 3h at room temperature before -80° C storage, with minimal impact on DNA quality and microbiome profile

If time to the lab cannot be <3h, we have recommended :

- freezing -20° C until -80° C storage ! NO freze-thawing !.

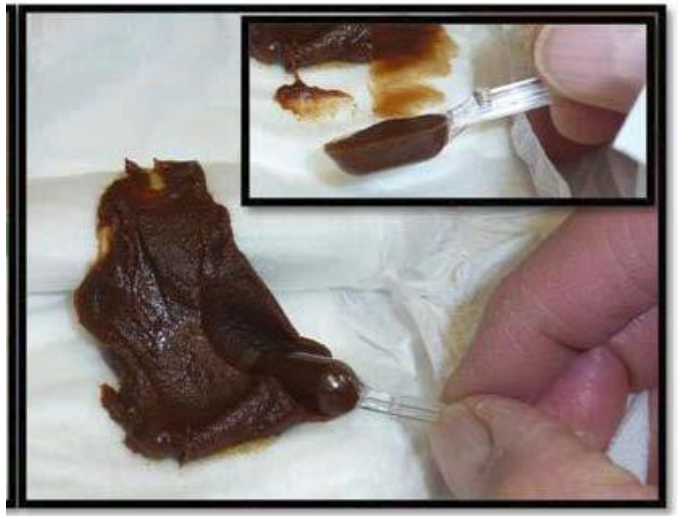
or

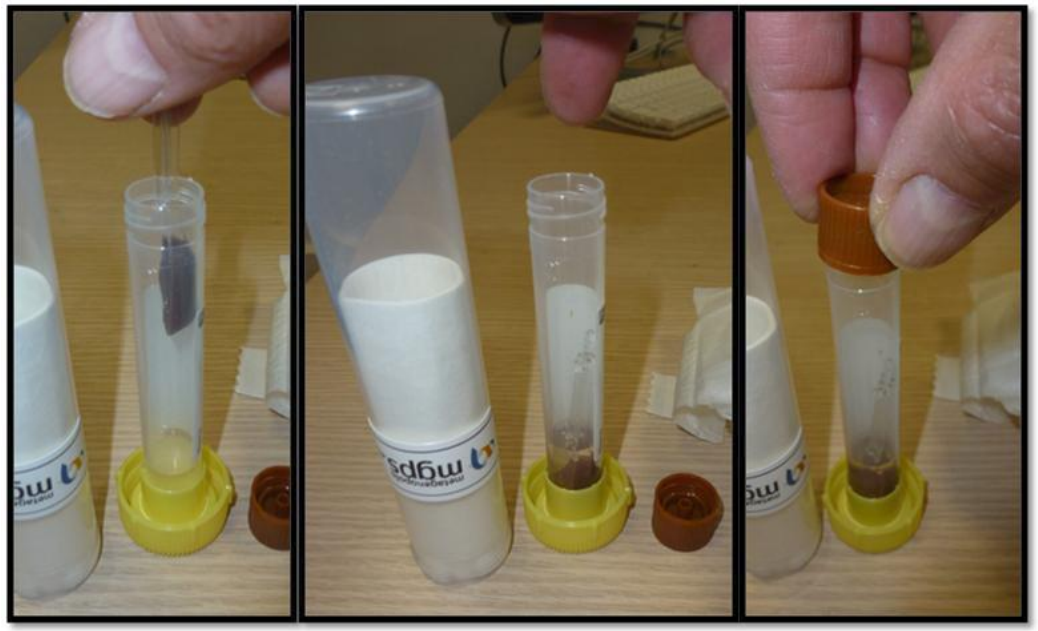
- Storage up to 24h at room temp

*** UF: Unfrozen stool show highly altered microbiome profiles**

Cardona et al, BMC Microbiol 2014

Stool collection in stabilizing solution

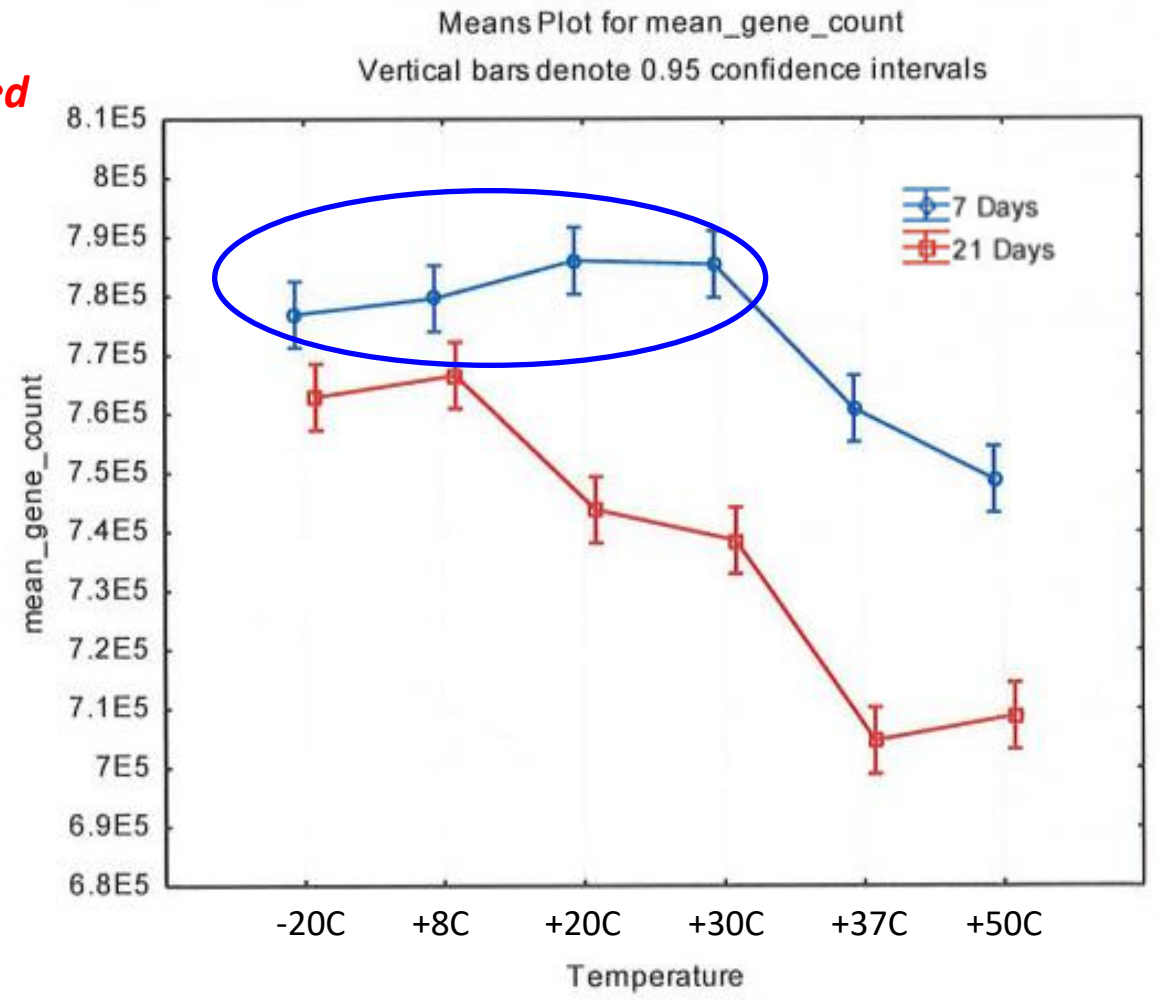




IHMS
SOP 05

Stability with time and temperature of stool collected in stabilizing solution

Unpublished



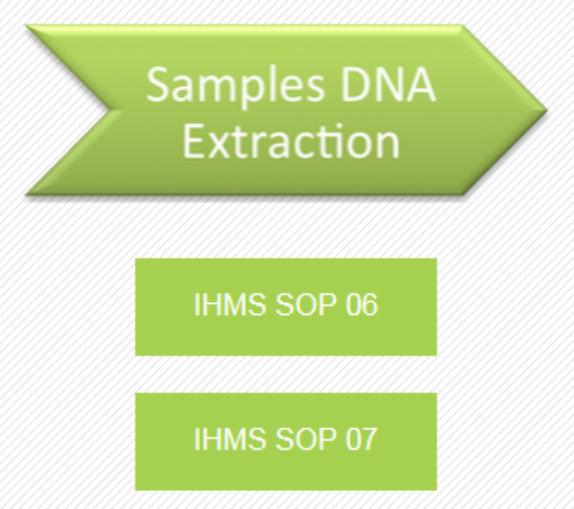
RNA-later stabilizes stool aliquots for 7 days at room Temp

Sample processing SOPs (DNA extraction)

International Human Microbiome Standards

2. Collection and processing of human stool samples

Dore, J., Ehrlich, S.D., Levenez, F , et al. and IHMS Consortium (2015)



2 fecal samples A & B ; 200 aliquots each

ROUND#1: 20 protocols (by their providers).

- 3 IHMS members & 17 invited participants
- 4 aliquots of each A and B
- 7 commercial kits & 3 automated systems

=> Top 5 protocols selected ; 3 nearly similar combined into 1 consensus (commercial kit)

ROUND#2: 3 protocols ; 4 participants (all 3 protocols) – inter-laboratory transferability

- 3 aliquots of each A and B

=> 2 protocols retained (SOPs 06 & 07)

Costea et al 2017 Nat Biotec

pre-analytical procedures - participant Labs

Legend: IHMS consortium members & WP2-associated participants

SCOTLAND (UK) : Aberdeen ; **IRELAND** : Cork

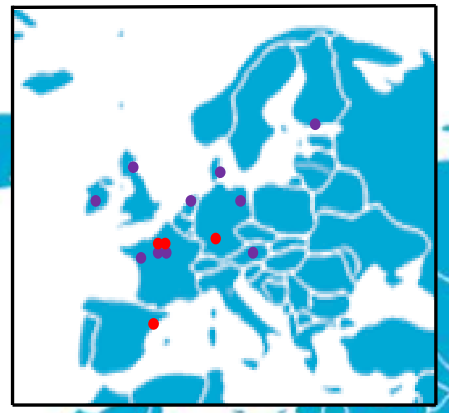
FRANCE :
Jouy-en-Josas – INRA Micalis1
Jouy-en-Josas – INRA Micalis2
Evry – CEA Genoscope
Palaiseau,
Nantes

SPAIN :
Barcelona - HUVH

CANADA :
London (ON)
Guelph (ON)
Vancouver

USA :
Houston (TX) – BCM
Chapel Hill (NC)
Kannapolis (NC)
Gainesville (FL)

FINLAND : Helsinki
DENMARK : Copenhagen
NETHERLANDS : Wageningen, Maastricht
GERMANY: Nuthetal
Heidelberg – EMBL
AUSTRIA: Graz



JAPAN :
Kanagawa

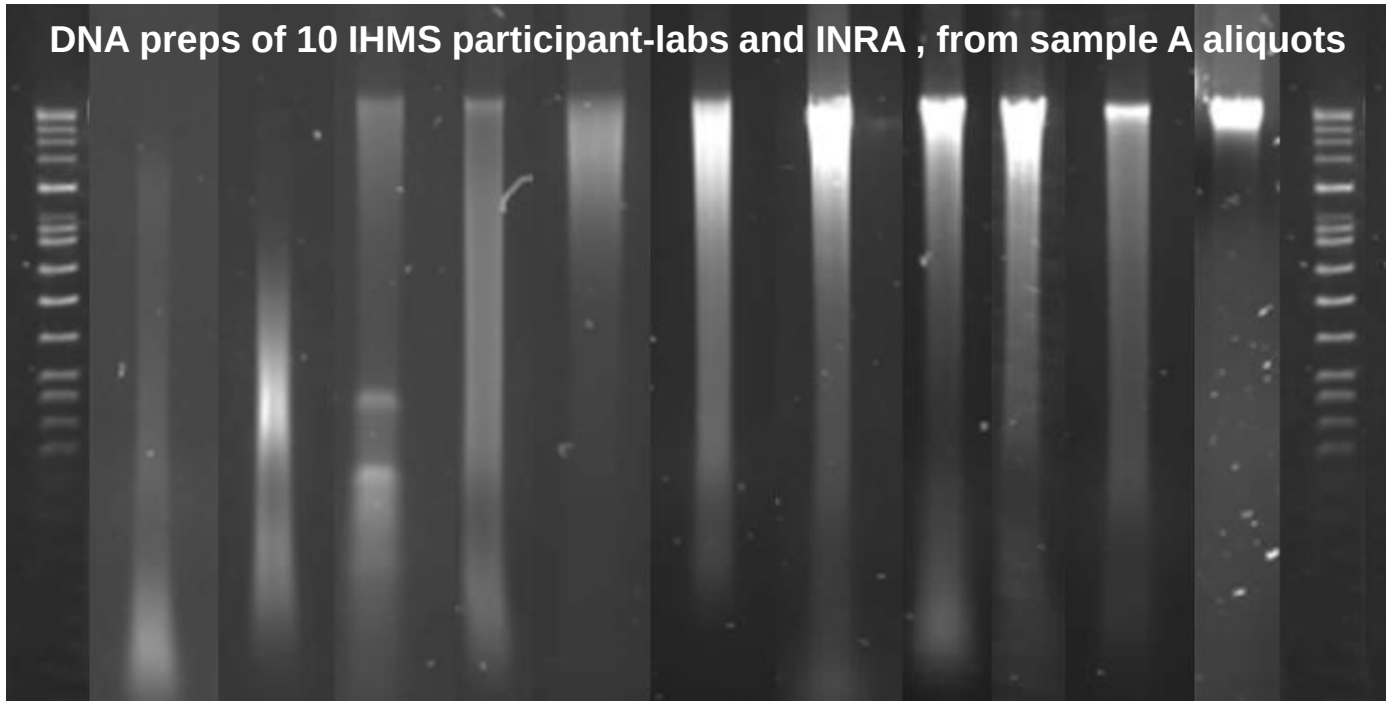
CHINA :
Shanghai - SJTU
Shenzhen - BGI

AUSTRALIA :
Brisbane

http://www.microbiome-standards.org

Rely on pipelines of standardized pre-analytical procedures for microbiome studies

DNA extraction : example of initial QC



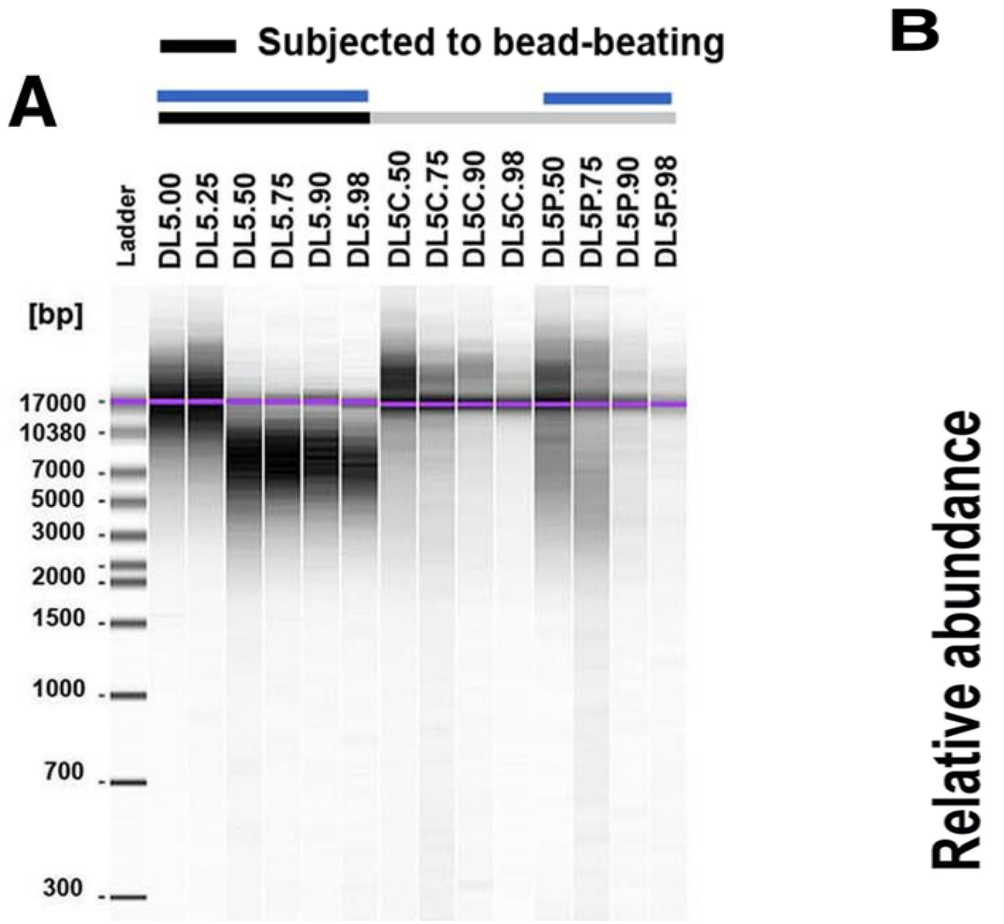
Note: gel figure reconstructed from selected lanes of 11 separate gels

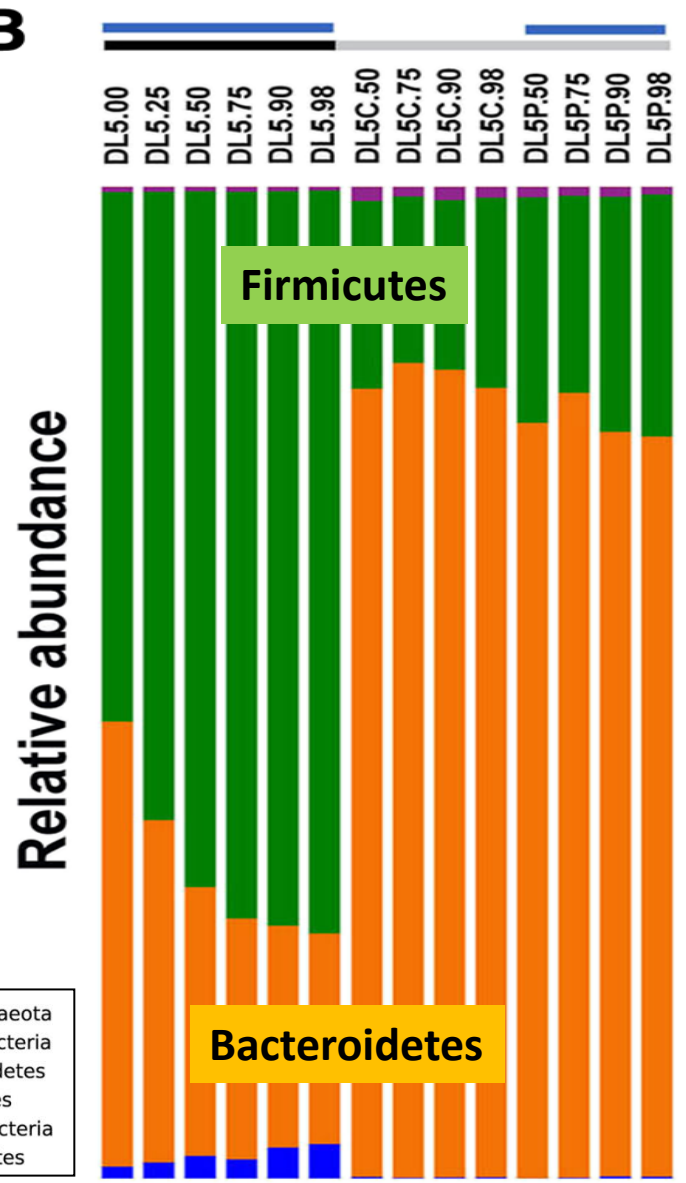
2.5 to 278 ng/µL with 82ng to 16µg DNA preparation ; from 150 mg aliquots (100 to 200 fold difference)

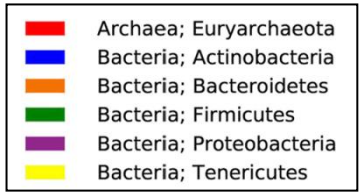
Costea et al 2017 Nat Biotec

http://www.microbiome-standards.org

Bead-beating is critical to lysis



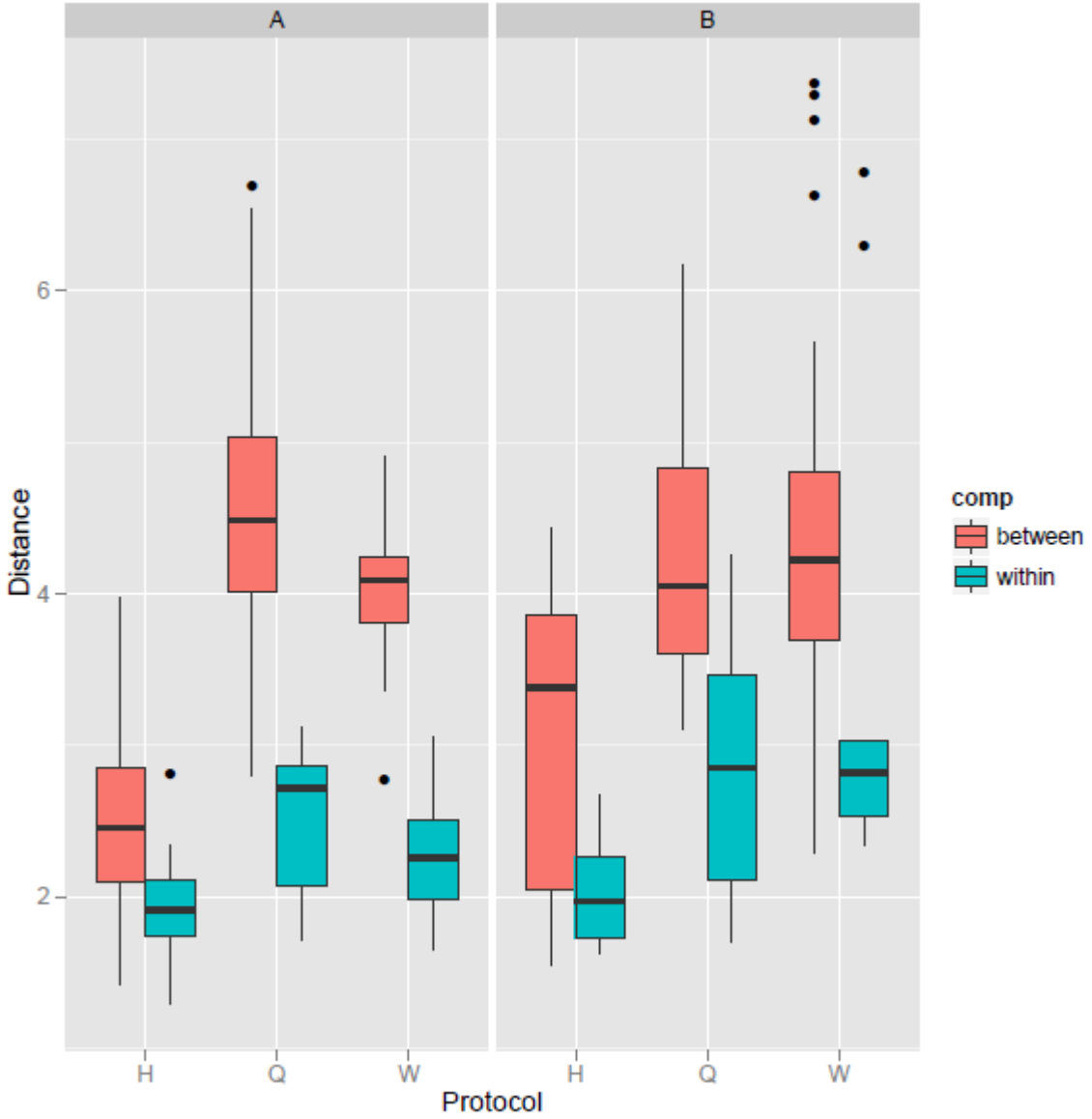




Bead-beating strongly impacts recovery of Firmicutes

Santiago et al, BMC Microbiol 2014

IHMS – dna-prep round#2 assessment



ROUND#2:
3 protocols H, Q, W compared based on within- and between protocols variation in composition :

H. least variations

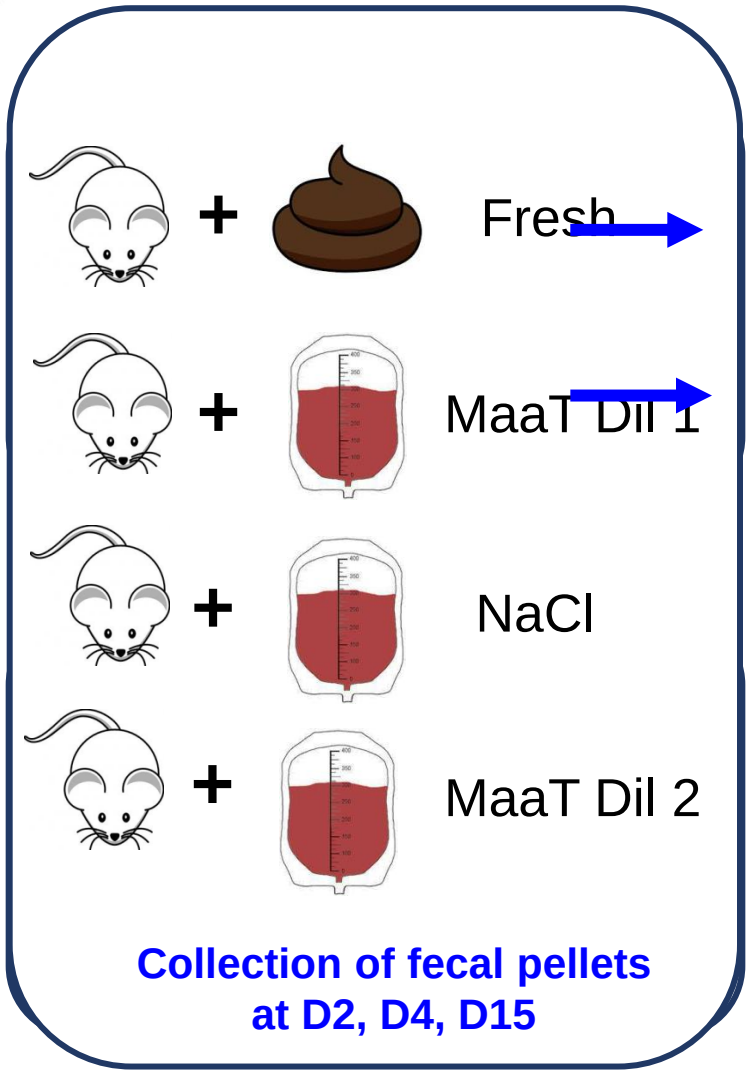
Q & W. high variations

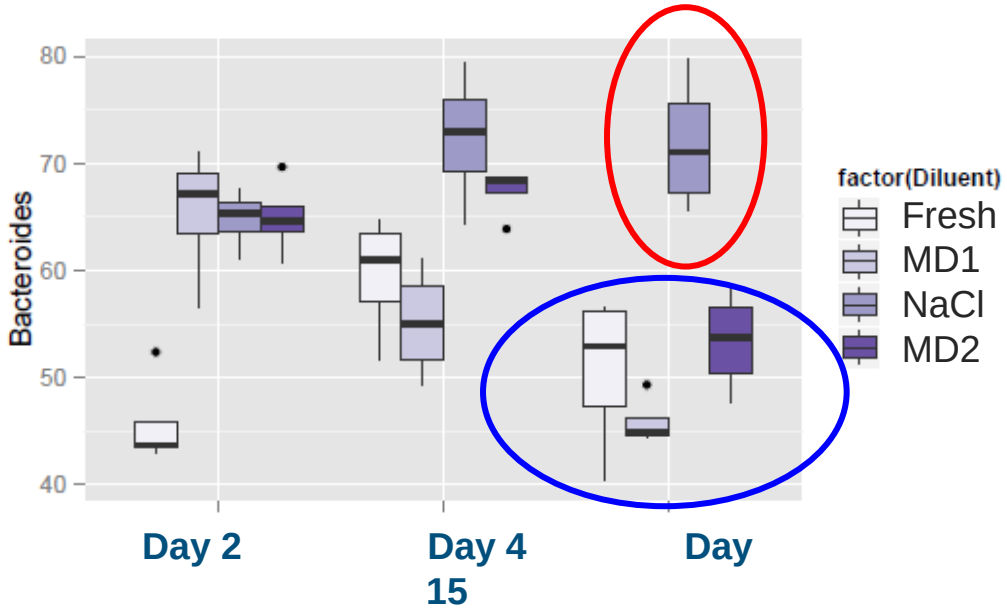
W. many outliers ;
difficult to implement

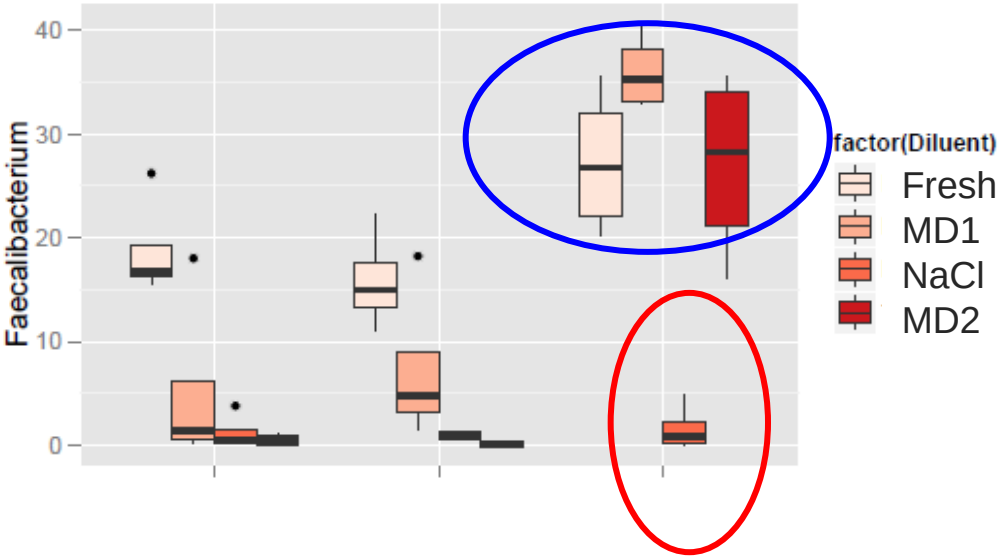
H & Q retained :
SOPs 06 & 07

Costea et al 2017 Nat Biotec

Rely on pipelines of standardized sample processing for microbiotherapy







www.MaaTPharma.com

Anticipate confounders in nutritional and clinical trials

Numerous elements interacting with the microbiome:

- drug therapy
  - Antibiotics, but also
  - Metformin
  - PPIs
  - transit modulators
  - Immune-targeted biologics
- diet
  - (pro/pre/syn/fiber…)
  - …

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  - Critical transitions rather than a health-disease continuum
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a large spectrum of microbiome studies…

- How ? : need for standards and major pitfalls to avoid
  - Implement rigorous pipelines relying on standardized procedures
  - Be prepared for complexity w inter-individual variations
  - Anticipate confounders
- **Resources you may use**…

Resources available :

Standard Operating Procedures @ http://www.microbiome-standards.org

stool collection and DNA extraction procedures ; analytics

Reference data (gene and species catalogs)

last updates may not be available but lots is there.

Ask expert laboratories for support

rather than trying to re-invent the wheel

Global joint efforts are still needed towards highest standards

Take home messages

- **the human is microbial, and the microbiome is a key driver of health and well being of the human holobionts**.

- **microbiomes differ** by genes, species, enterotypes (ecology) and gene count (microbiota diversity) ; large studies are necessary

- **dysbiosis is an altered state of host-microbes symbiosis** hence accounting for this complexity will be essential as we explore mechanisms and aim at designing new tools

- **standardization is crucial to implement microbiome studies in the clinical setting**

- **the microbiome is sensitive and responsive to many confounders**

nutrition and live microbes will be strategic bioactives for the maintenance, preservation or restoration of man-microbes symbiosis

- **microbiome studies** in the clinical setting **are essential for the translation** of corresponding innovations and towards personalization.

- prescription of **microbiome assessment in general population and clinical practice will be the next frontier**

Merci de votre attention !

Questions ?

Joel.dore@inra.fr

Session 2: Mesures quantitatives et qualitatives des microbiotes, validations et normes européennes et internationales

Coordinateurs : Joël DORÉ, *INRA*, David PETITEAU, *INRA* et André TORDEUX, *Genoscreen*

Table Ronde.

Françoise Le Vacon, *BIOFORTIS Research*, Stéphanie Ferreira , *Genoscreen*, Pierre Rimbaud , *Enterome*

Sous-TR1 Standardisation _ *L'analyse des microbiomes en général fait face à des difficultés techniques !*

1a- standardisation de la qualité, de la technique elle-même.

Quels sont les biais ? Est-ce qu'on a des process qualité bien acquis ?

1b- méthodo biostat specifications.

Quelle place de la métagénomique et autres omics ? Informations et sensibilités

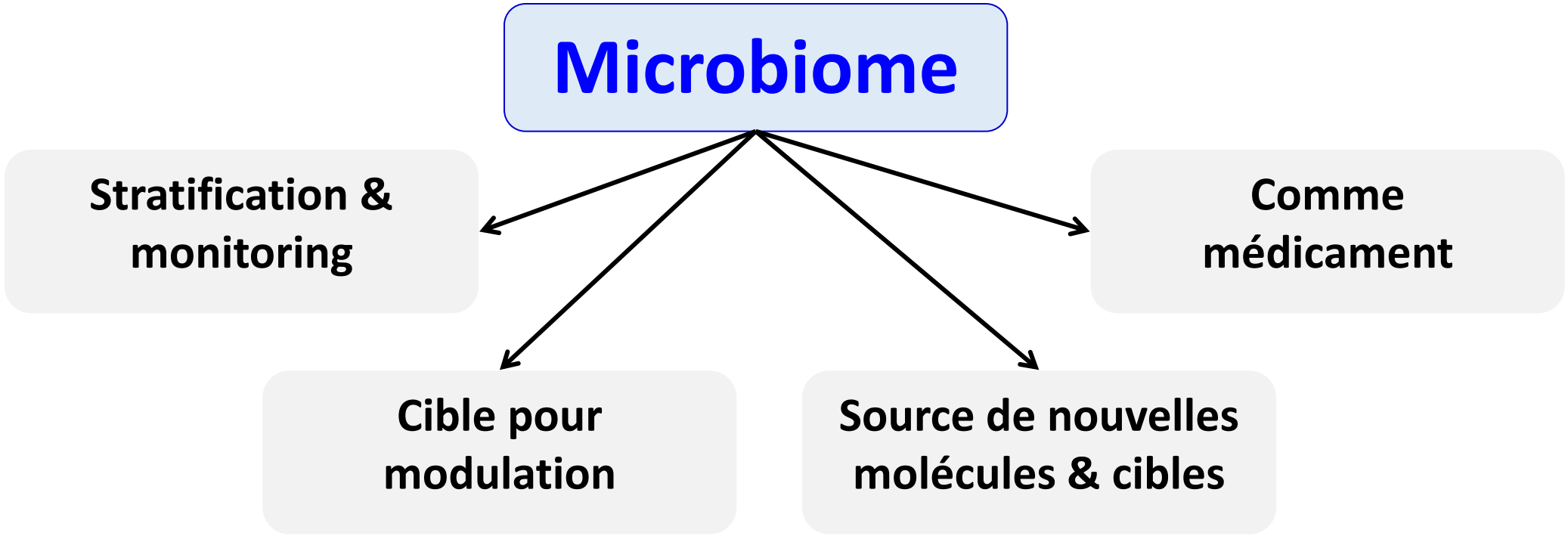
Sous-TR2 Applications cliniques _ *ces techniques standardisées en médecine / en pratique ?*

2a- à quoi peuvent s'appliquer ces méthodes aujourd'hui et demain ?

Qu'est-ce qu'il faut faire pour avoir des 'applis' utiles ?

2b- validation clinique. Quel chemin vers les applications pratiques ?

Microbiome : cible ou atout ?



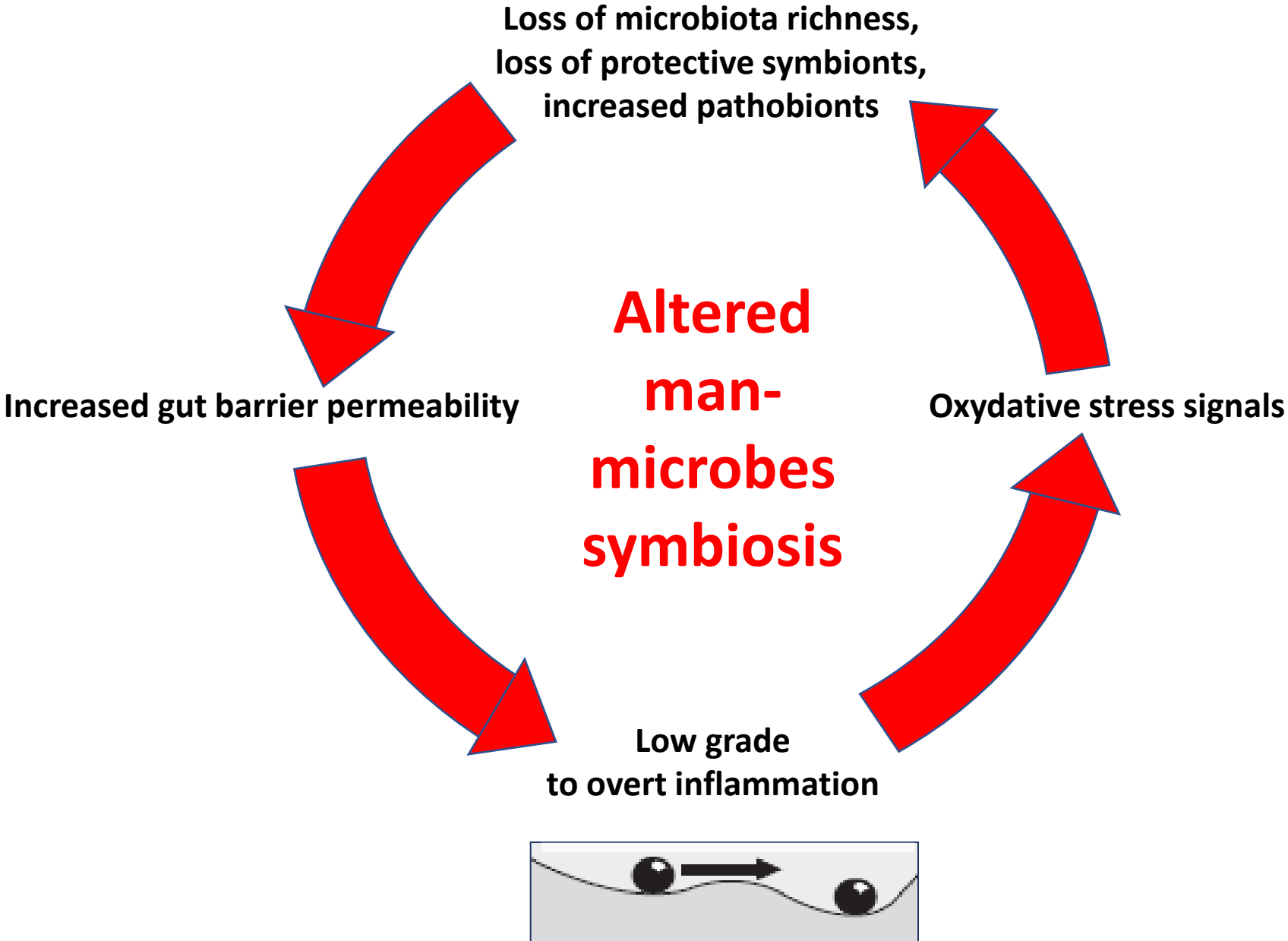
ELSEVIER
Available online at ScienceDirect www.sciencedirect.com
Elsevier Masson France EM|consulte www.em-consulte.com

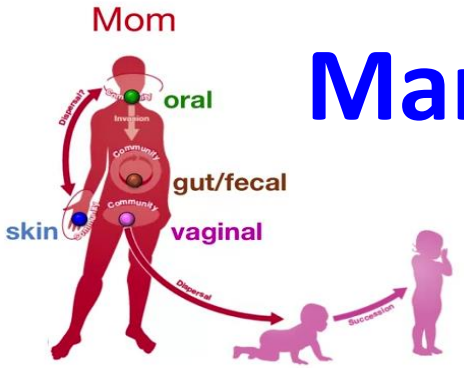
GIENS WORKSHOPS 2016 / *Translational pharmacology* *Thérapie, 2017. 72:21-38*

The human gut microbiome as source of innovation for health: Which physiological and therapeutic outcomes could we expect?☆

Sanofi, Takeda, MaaT Pharma, Hôpital Bichat, Astra Zeneca, Hôpital Cochin, Institut Mérieux, Enterome, Merck Sharpe&Dohme, Inserm, Institut Pasteur, Pharmabiotic Research Institute, Ipsen, Leem/ARIIS, HôpitalSaint-Louis - APHP, Bioaster, AvieSan, Nestlé, INRA

Circular causalities in immune-mediated diseases





adapted from Gonzalez et al. 2011, EMBO reports

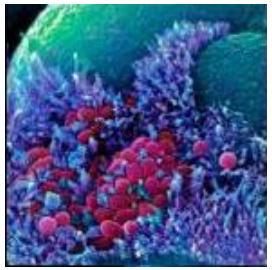
Man-Microbe symbiosis

Homo sapiens 'symbiosus' ;

a man-microbe mutualism that starts at birth

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 : Risk of infection

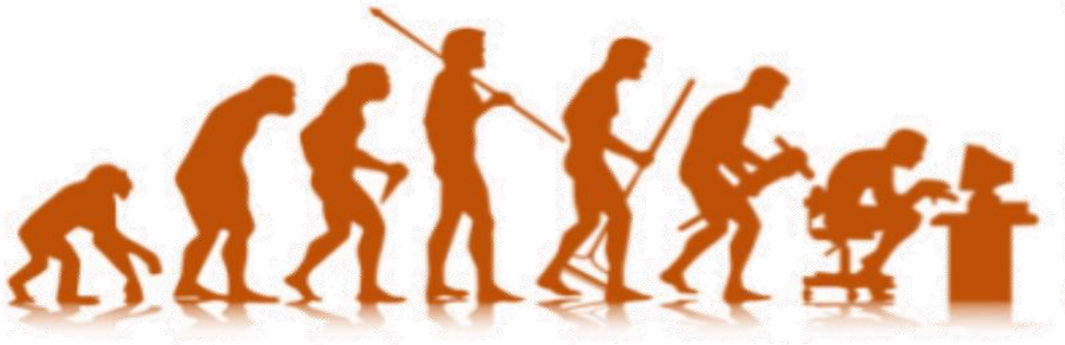
Disruption of tolerance : Risk of immune-mediated disorders

We want to monitor, diagnose, predict, restore, for a preventive nutrition and a medicine of *Homo symbiosus*

Over the passed 2-3 generations …

We dramatically changed

- ✓Birth modalities and environment
- ✓Nutrition & activity
- ✓Exposure to xenobiotics



neglecting we are microbial

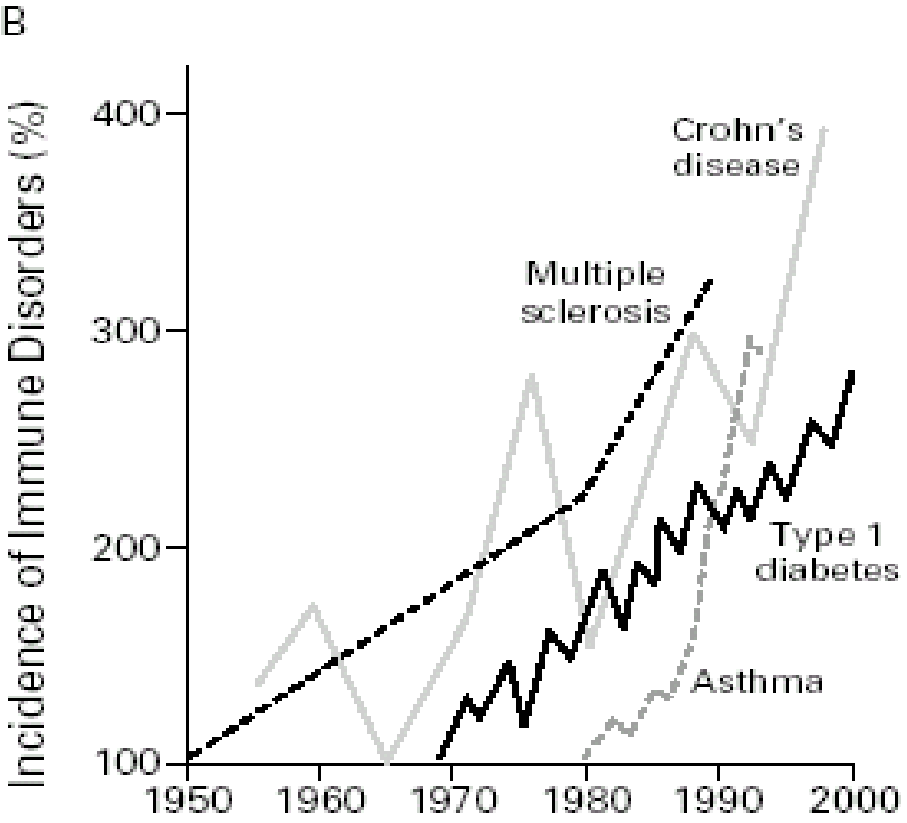
- ✓~ 50% bacterial cells in numbers
- ✓> 1 kg microbial biomass
- ✓~ 70% unknown (non cultured)

Chronic diseases increased in incidence over the past 60 years

1 person in 4 affected by 2025

No PREVENTION!
No CURE!

Evidence linking to … altered microbes-host symbiosis



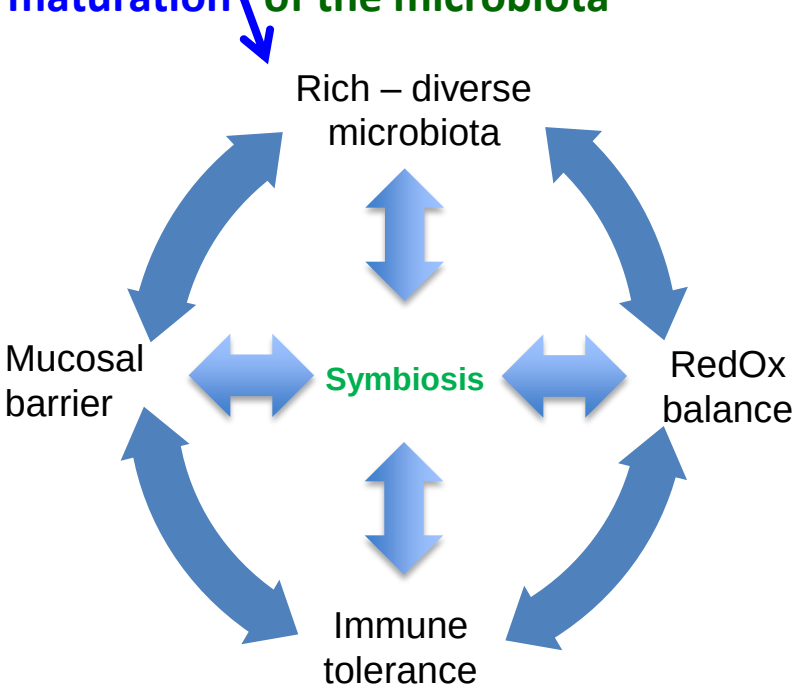
Bach JF, N Eng J Med 2002

Man-Microbes symbiosis

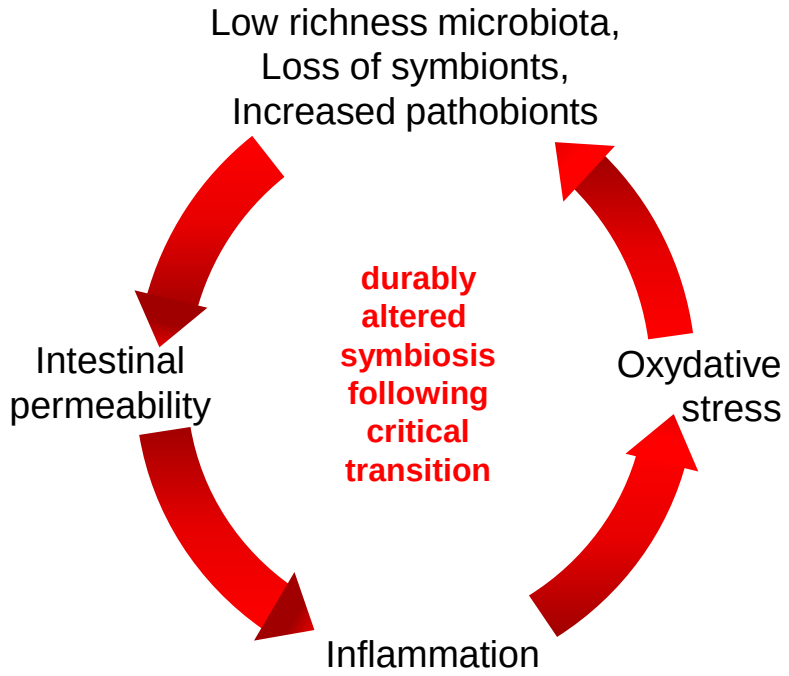
Homo 'symbiosus'

to *Homo 'dysbioticus'*

Immune maturation & development of the microbiota



Altered symbiosis :
- **nutrition**
- **antibiotics**
- **xenobiotics**
- **……..**



Van de Guchte, Blottière, Doré. Microbiome 2018

alteration of human-microbes symbiosis comes with recurrent features

Crohn's disease Mangin 2004, Manichanh 2006, Sokol 2008, De Crutz 2012

Low species richness ; reduced proportions of symbionts *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and increased pathobionts...
on the microbiome side

Altered gut-barrier permeability, low grade inflammation and oxydative stress
on the host side

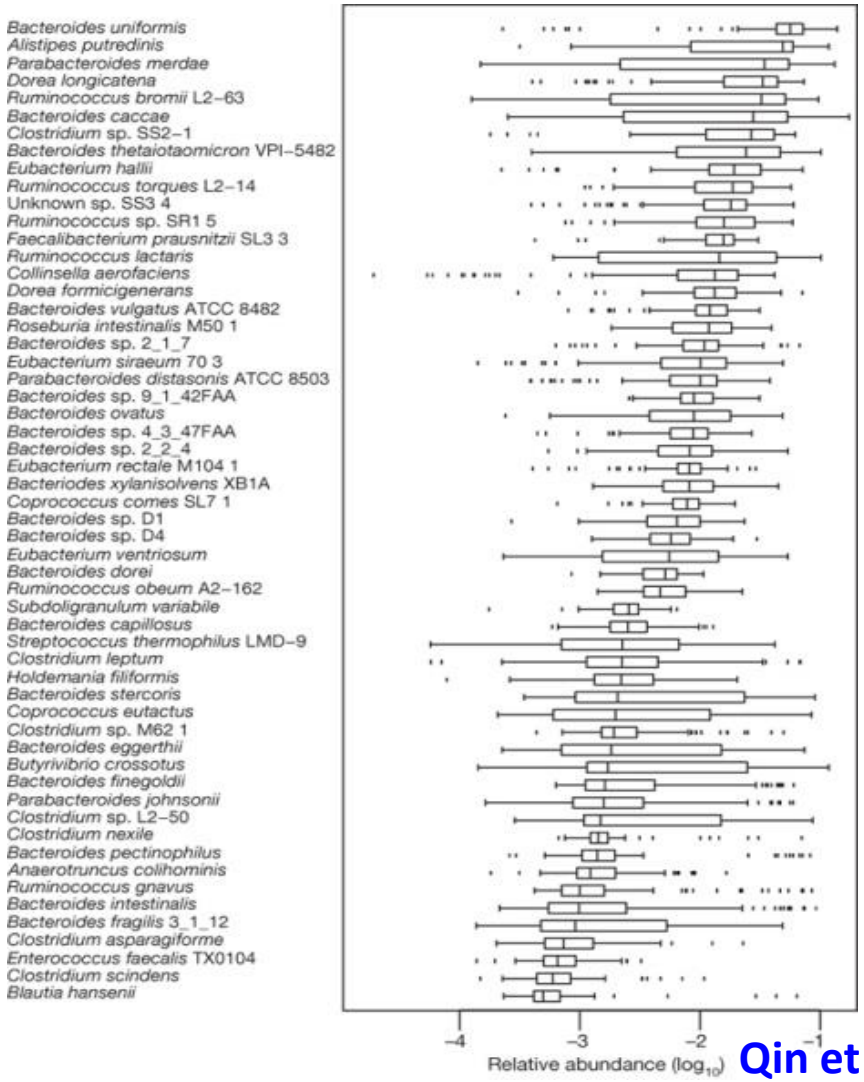
Cystic ..., 2002, ...012, Del Campo 2014,

AAD *C.difficile* Rea Mary 2012

Indications from animal models, effects of antibiotics or probiotics, clinical studies; …

Be prepared for complexity with high inter-individual differences

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

Qin et al. Nature 2010

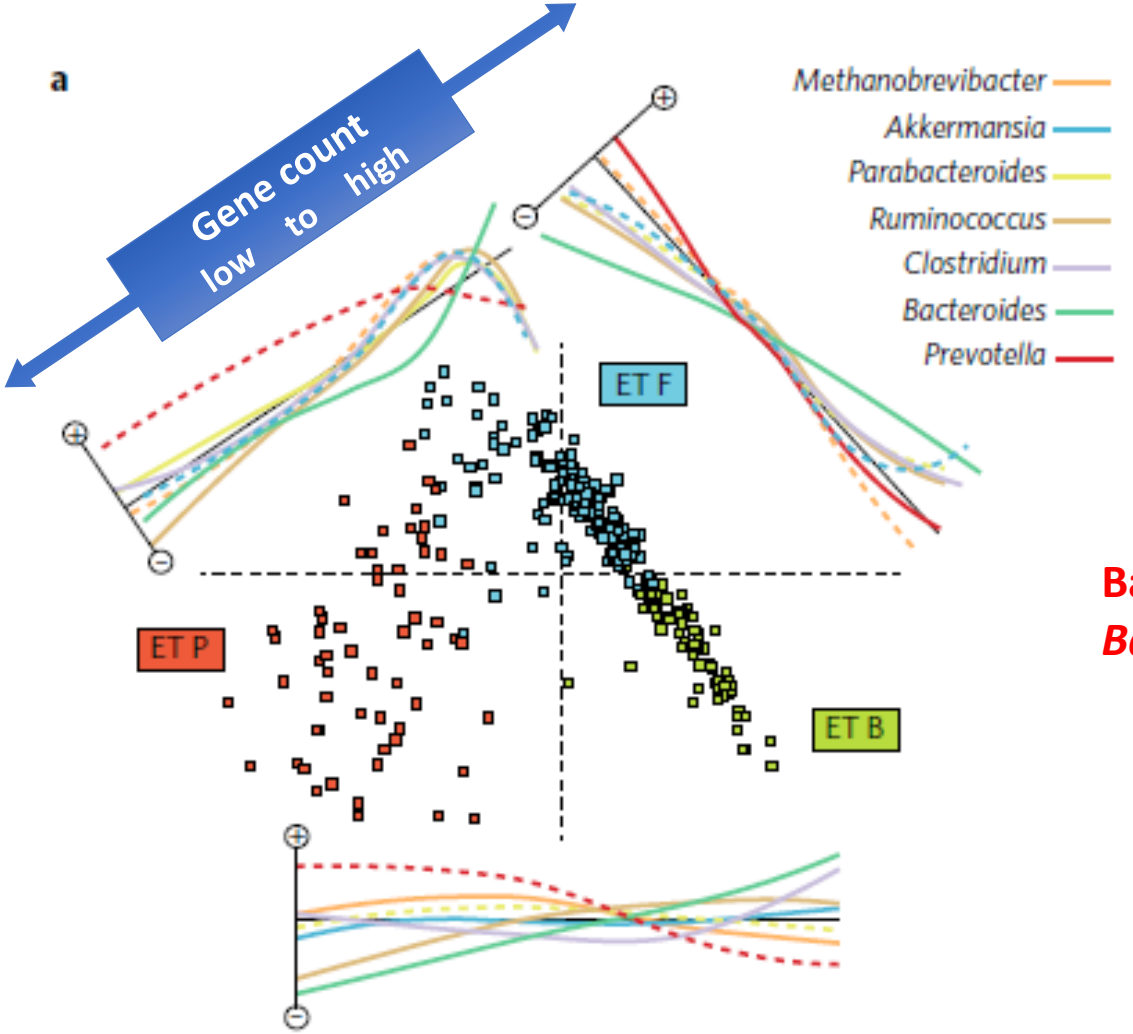
Today:
> 10 million genes
> 1500 metagenomics species

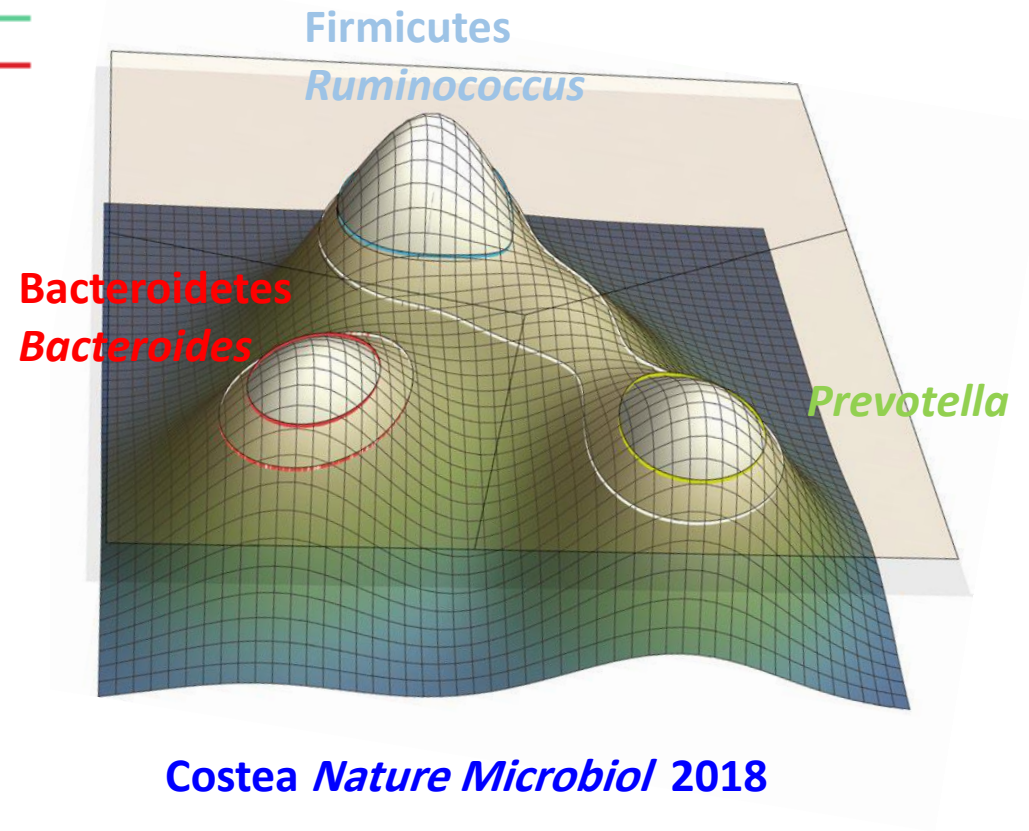
Hence microbiome pattern is highly subject specific

Li et al. Nature Biotech 2014

Complexity with enterotypes as a stratifier of microbiomes:

preferred patterns within the landscape of all possible arrangements





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

also observed in monkeys, pigs, mice…

Complexity with gene-count as a stratifier of microbiomes:

Low gene count and High gene count microbiomes behave different