Human intestinal microbiota in health and disease
- a microbiomics perspective -

Joël Doré
INRA, Micalis, Jouy-en-Josas, France
Integrated microbiomics view:

Diversity, composition → Metagenome → mRNA → Metaprotome → Metabolome → Host
Species diversity profiling indicates high inter-individual variations ...

14 healthy adults

Only a few bacterial species appear conserved

2 healthy adults

...and stability over time

Zoetendahl et al. 1998; Sutren et al. 2000

Seksik et al. 2003; Vanhoutte et al. 2004
**Bacteria/g feces**

**Dominant microbiota**

**Sub-dominant microbiota**

**Transiting Bacteria**

**Phylogenetic groups and spp (qPCR assessment)**

- 10^12 Bacteria
- 10^11 C. Leptum group
- 10^10 C. Coccoides group
- 10^9 Bacteroides Prevotella group
- 10^8 Bifidobacterium
- 10^7 Enterococcus
- 10^6 L. rhamnosus
- 10^5 L. lactis
- 10^4 L. casei
- 10^3 S. thermophilus
- 10^2 L. plantarum

**Species**

- L. rhamnosus
- L. lactis
- L. plantarum
- S. thermophilus
- L. casei

**J-P Furet et al., 2009**
• Colonisation and immune imprinting - early times -
• Early colonisation and geographical location
Maternal milk, source of active immune imprinting (n=7 mother-newborn couples)

Molecular signatures shared by different biological fractions
⇒ Potential transfer from mother to newborn via maternal milk
⇒ *Streptococcus salivarius*, *Staphilococcus epidermidis*, *Bif. longum*

P. Perez et al. 2007 Pediatrics
Infant fecal microbiota differs from that of adults, until 2nd year of life. 

**Genus Bifidobacterium** is most represented

**Bacteroides and relatives** are early colonizers following enterobacteria.

Adult phylogenetic groups **C. coccoides and C. leptum** are not ‘yet’ dominant.

M. Fallani et al., JPGN 2010
Birth location has an incidence on composition of the fecal microbiota.

Nord-South gradient from Stockholm, Sweden to Granada, Spain: more bifids in the north; early diversification in the south

M. Fallani et al., JPGN 2010
the dominant fecal microbiota of elderly
-specific alterations at the level of dominant species-

Under-represented: Faecalibacterium sp.

Over-represented:
- Oxalobacter
- Akkermansia
- Bifodobacterium

Montecarlo test
P = 0.024

Elena Biagi – Patrizia Brigidi – Claudio Francesci - University of Bologna
Lotta Nylund, Reetta Satokari & Willem M. de Vos University of Helsinki & Wageningen
Core microbiome of the « normal » microbiota of healthy subjects ; a schematic view

Core species
2% of non-redundant

Core genes
9% of non-redundant

Diversity, composition
Metagenome
mRNA
Metaprotome

Host
inter-individual redundancy within the intestinal ecosystem at the level of environmental proteome?
Metaproteome of human intestinal microbiota
Pilot study in 6 volunteers / cytosolic proteins of fecal bacteria

1092 proteins common to both subjects
397 proteins specific to subject 1
376 proteins specific to subject 2
1865 validated spots ~ 59 % comigrating spots * for 2 metaproteomes

Juste et al, unpublished

* up to 15% may be host proteins
Core microbiome; a schematic view

- **Diversity, composition**
- **Metagenome**
- **mRNA**
- **Metaprotome**

Core species: 66/>1000
Core genes: 1,000’s / 3M
Core proteins: 100’s />2000
Eubiosis versus Dysbiosis

Defining “normal” microbiota - **Eubiosis** - : density, diversity, composition, core-species, dynamics (stability and resilience) of structure and functions (core-microbiome),

Hence, it becomes possible to explore **Dysbiosis** of the dominant intestinal microbiota in patients compared to healthy subjects.

This opens novel fields of exploration:
- rationality for strategies to restore Eubiosis / homeostasis.
- mechanisms by which dysbiosis of the intestinal microbiota acts in chronic, immune, metabolic or degenerative pathologies; especially in the context of **bacteria-cell crosstalk**...
## Indicators of dysbiosis in disease versus health

<table>
<thead>
<tr>
<th>Disease</th>
<th>suspicion for an involvement of the commensal microbiota</th>
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</thead>
<tbody>
<tr>
<td>Frailty in seniors</td>
<td>Van Tongeren et al., 2005</td>
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<tr>
<td>Crohn</td>
<td>Seksik et al., 2003; Sokol et al., 2006, 2008, 2009; Qin et al., 2010</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Sokol et al., 2008; Martinez et al., 2008 Hutson unpublished</td>
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<tr>
<td>Pouchitis</td>
<td>Lim et al., 2009, Kühbacher et al., 2006</td>
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<tr>
<td>Obesity</td>
<td>Ley et al., 2007; Kalliomäki et al., 2008; Furet et al. 2010</td>
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<tr>
<td>Type-2 diabetes</td>
<td>Cani and Delzenne, 2009 ; Sun et al., 2010</td>
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<tr>
<td>Type-1 diabetes</td>
<td>Dessein et al., 2009; Wen et al., 2008</td>
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<tr>
<td>Coeliac disease</td>
<td>Nadal et al., 2007; Collado et al., 2009</td>
</tr>
<tr>
<td>Allergy</td>
<td>Kirjavainen et al., 2002; Björkstén, 2009</td>
</tr>
<tr>
<td>Autism</td>
<td>Finegold et al., 2002; Parracho et al., 2005</td>
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</table>
Importance of microbiota in immune diseases increasing in prevalence?
INCIDENCE OF CROHN’S DISEASE

Cases per 100,000 persons-year

Data from Ekbom et al, in McDermott & Stenson ‘IBD’
PEDIATRIC CROHN’S DISEASE IN SCOTLAND

Cases per 100,000 persons-year

Sawcenko et al, Lancet 2001
EPIDEMIOLOGY OF IBD: GENETIC SUSCEPTIBILITY

• First-degree relatives: RR 10.7 for IBD

• Concordance rates for IBD in twin pairs
  for CD
  monozygotic twins: 33-50%
  dizygotic twins: 0-10%

  for UC
  monozygotic twins: 13-19%
  dizygotic twins: 0-5%

Inflammatory Bowel Diseases
- Crohn’s disease
- Ulcerative colitis

Dysbiosis of the Gut Microbiota?

Genetic Predisposition

Deregulation of the Immune Response
MUCOSA ASSOCIATED MICROBIOTA IN IBD

Ott et al,
GUT 2005
Higher bacterial concentrations in mucosa associated microbiota of CD patients
Reduced Microbial Gene Diversity in IBD
Fecal microbiota dysbiosis in Crohn’s disease

Healthy library

Bacteroidetes
371 clones
33 OTUs

Proteobacteria
25 clones
5 OTUs

Actinobacteria
50 clones
7 OTUs

Crohn library

Bacteroidetes
371 clones
33 OTUs

Proteobacteria
25 clones
5 OTUs

Actinobacteria
50 clones
7 OTUs

Firmicutes
37 clones
13 OTUs

Clostridium coccoides group

Clostridium leptum group

Bacteroides
356 clones
33 OTUs

Firmicutes
89 clones
43 OTUs

Clostridium
coccoides group

Manichanh et al, Gut 2006
Fecal and mucosal microbiota dysbiosis in Crohn’s disease

Lesser proportion of bacteria from the *Clostridium leptum* group in CD

Manichanh et al., Gut 2006, Sokol et al., IBD 2006

Harry Sokol
Mucosal Dysbiosis in Crohn’s Disease

Ph. Marteau & Ph. Pochart

20 patients with active CD, requiring ileo-caecal resection:

- M0 surgical resection
- M6 colonoscopy

FISH analysis of biopsies

- Eub338 (Eubactia)
- Bac303 (Bacteroides-Prevotella)
- Ent1458 (Enterobacteria)
- Erec482 (Clostridium coccoides)
- Lab158 (Lactobacillus-Enterococcus)
- Bif164 (Bifidobacterium)
- Fprau645 (F. prausnitzii and relatives)

Still in remission or Endoscopic relapse

Harry Sokol, Philippe Langella et al. PNAS 2008
Mucosal Dysbiosis in Crohn’s Disease
Ph. Marteau & Ph. Pochart

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- M0 surgical resection
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- Fprau645 (F. prausnitzii and relatives)

F. prausnitzii at M0 (p=0.027)
3.3% → Remission at M6
0.3% → Relapse at M6

Faecalibacterium prausnitzii is associated with protection from endoscopic inflammation relapse 6 months after surgery ⇒ anti-inflammatory properties
Faecalibacterium prausnitzii;

A commensal bacterium with anti-inflammatory properties in vitro and in vivo

Sokol, et al. PNAS 2008

Willing et al. IBD 2009 [dysbiosis: F prausnitzii -; E coli + in ICD]
Anti-inflammatory properties of *F. prausnitzii*

1. **In vitro experiments:**
   - High IL10/IL12 cytokine release by Peripheral blood mononucleated cells
   - Reduction of IL-1β induced IL-8 secretion by Caco-2 cells
   - Supernatant abolished TNFalpha induced NF-kB activity in HT-29 cells

2. **In vivo experiments:**
   - Both *F. prausnitzii* and its supernatant reduced scores and blood parameters of inflammation in TNBS-induced colitis in Balb/c mice
   - Administered IP, its supernatant protected mice from death induced by TNBS.

*Sokol et al. PNAS 2008*
TNBS induced Colitis: intraperitoneal administration of *F. prausnitzii* fractions

*Faecalibacterium prausnitzii* supernatant contains “bioactives” that confer protection against a lethal condition caused by TNBS induced gut inflammation.
qPCR validation of fecal microbiota dysbiosis (phylogenetic µarray) in Crohn vs healthy subjects

Seungha Kang, Chris McSweeney, Mark Morrisson
Profiling-based assessment of fecal dysbiosis (PCR-DGGE) in Crohn patients vs healthy subjects

Several candidate marker-species differed in prevalence and relative intensities (P<0.05) and *F. prausnitzii*, *B. adolescentis* and *R. gnavus* were validated by qPCR

M. Joossens et al. submitted
RT-qPCR validation of fecal microbiota dysbiosis (metagenome) in Crohn patients vs healthy subjects

- **O. valericigenes** is detected in 12% of Crohn patients vs 93% of controls, and with a higher abundance in controls than in CD patients (Δ Ct = 17.33, P<0.001)

S. Mondot et al. *IBD 2010*
Fecal microbiota dysbiosis (µarray) in Crohn patients vs healthy subjects (n=14+16)

S. Mondot et al.
IBD 2010
Fecal Dysbiosis in Crohn’s Disease
at the level of metagenome

p-value: 0.031

Francisco Guarner (Val d’Hebron Hospital, Barcelona)
Wang Jun and colleagues (BGI-Shenzen)
Dusko Ehrlich, Patricia Lepage, Julien Tap (INRA)

Junjie Qin et al Nature, March 2010
Fecal Dysbiosis in Crohn’s Disease
at the level of metagenome: CD related genes can be identified.

- A cohort of 21 Spanish individuals
- patients: n = 8
- healthy: n = 13

- Ranksum search for genes different in frequency (hits/kb/30 million reads) between the two groups in a 3.3 million gene catalog

3802 “CD related genes” found at p< 3*10^{-4}

Ehrlich et al.
Fecal Dysbiosis in Crohn’s Disease
at the level of metagenome: CD related genes can be identified.

Phenotype | CD genes (median) | “Gene loss” in CD
Healthy   | 3038 out of 562 271 | p<2*10^{-13}
CD        | 643 out of 425 397 | (one-tailed t test)

Ehrlich et al. => diagnostic model with a limited set of marker-species (5 Firmicutes candidates)
**Metaproteomic signatures in Crohn**

12 participants (age & sex matched)
6 Crohn’s Disease patients (CD) vs 6 healthy controls (HC)

Differential protein expression from 2D-DIGE experiments
- 39 under-expressed in CD
- 41 over-expressed in CD

Protein spot identification using LC-MS/MS, in progress:
- 66 spots identified out of 84 already analysed (~78% yield)
- **over-expressed proteins** associated with *Bacteroides* (several known as “immunoactive”, and/or with eucaryotic homologs)
- **under-expressed proteins** mainly associated with Firmicutes

Catherine Juste & Saint-Antoine Hospital
INRA platforms PICT & PAPSSO
Metabolomic signatures in Crohn

12 volunteers: 6 healthy vs 6 Crohn


Tyrosine metabolism, amino-acids, bile acid metabolism, ...

**Bile acid metabolism**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>ICD</th>
<th>CCD</th>
<th>Healthy</th>
<th>P value concn. ICD vs others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocholate</td>
<td>5/6</td>
<td>4/8</td>
<td>0/10</td>
<td>.001</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>5/6</td>
<td>2/8</td>
<td>2/10</td>
<td>.002</td>
</tr>
<tr>
<td>Glycochenodeoxycholate</td>
<td>5/6</td>
<td>2/8</td>
<td>1/10</td>
<td>.002</td>
</tr>
</tbody>
</table>
Dysbiosis in Crohn’s disease => vicious circle favoring aggravation and chronicity

- increased bacterial density at mucosal level (Swidzinski 2002)
- increased proportion of immuno-agressive commensals (Gram-negative, subdominant in health?) (Darfeuille Michaud 2004, Sokol 2009, Willing 2009)
- reduced proportion of anti-inflammatory commensals (Gram-positive Firmicutes?, Actinobacteria?) (Sokol 2009, Willing 2009)
- increased proportion of proteins potentially promoting auto-immunity (Juste submitted)

Further contributions of Dysbiosis: disruption of tolerance, alteration of mucosal healing, secretion of defensins, … ?
Complex interactions underlying polygenic obesity

Exercise

Nutrition

Viruses

Social Status

Allergies

Food Abundance

Peer pressure

Pollution

Psychology

GUT Microbiota

Endocrine gland

Mutch D & Clement K, Plos genet 2006
Transcriptional interaction networks in human adipose tissue

Overexpressed in obese AT

- Leukocyte transendothelial migration 5.5%
- Cell adhesion molecules (CAMs) 5.5%
- Small cell lung cancer 5.5%
- Hematopoietic cell lineage 9.1%
- ECM-receptor interaction 9.1%
- Regulation of actin cytoskeleton 11.8%

Underexpressed in obese AT

- Lysine degradation 1.8%
- Ribosome 10.9%
- Adipocytokine signaling pathway 13.6%
- Fatty acid metabolism 13.6%
- Oxidative phosphorylation 13.6%

Increased inflammation

Altered metabolism

Henegar, Genome biology, 2008
Prifti, Bioinformatics, 2008
Bypass surgery Switch of functions / weight loss (3mo)

Overexpressed themes

- Chronic myeloid leukemia: 9.7%
- Prostate cancer: 9.7%
- B cell receptor signaling pathway: 10.4%
- T cell receptor signaling pathway: 10.4%
- Colorectal cancer: 11.1%
- Wnt signaling pathway: 12.5%
- ErbB signaling pathway: 13.9%
- Jak-STAT signaling pathway: 16.7%
- MAPK signaling pathway: 23.6%
- Cytokine-cytokine receptor interaction: 27.8%

Underexpressed themes

- Glycosaminoglycan degradation: 8.2%
- Glycan structures - degradation: 12.3%
- Aminoacyl-tRNA biosynthesis: 12.3%
- Ribosome: 23.3%
- Purine metabolism: 24.7%
- Oxidative phosphorylation: 27.4%

Improved inflammatory context

Improved
Metabolism
Tissue remodeling

Transcriptional domain cover
Metabolism & inflammation improvement after GBP

**Calorie intake (kcal)**

-50%

**Leptin (ng/ ml)**

-50%

**Weight (kg)**

-22%

**Glycemia (mmol/l)**

**hsCRP (mg/dl)**

Wilcoxon pairé; * p<0.05 vs M0 ; * entre M3 et M6
**Bacteria groups increasing with weight loss**

Amount of bacteria = \( \log_{10} (\text{bacteria}) - \log_{10} (\text{total bacteria}) \)

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**Bacteroides/ Prevotella**

Variation associates (- neg) with corpulence (BMI, FM, Leptin)

But dependant on calorie ( LME).

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**E. coli**

Phylum: *Protéobactéries*

Associations (-ve) with BMI, FM, Leptin (R -0.53  \( p < 0.001 \))

Remained if calorie adjusted

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Furet JP, Kong LC et al  *Diabetes* 2010

Wilcoxon, * \( p<0.05 \) vs C; Wilcoxon pairé, * vs M0
Bacteria groups decreasing with weight loss

Bifidobacterium

*Phylum: Actinobactéries*

Lactobacillus

*Phylum: Firmicutes*

Positive correlations (+pos) with BMI, % fat mass, Leptin, Insulin (R 0.30, P<0.05) / depend on calorie intake (adjustments)

_Furet JP, Kong LC et al_ Diabetes 2010

Wilcoxon paired, * vs M0
Bacteria groups associate with low-grade inflammation

F. prausnitzii

C. leptum; Phylum: Firmicutes

F. prausnitzii is lower in diabetics

Negative correlations with inflammatory markers: basal & kinetics

hsCRP (R -0.39), Interleukin 6 (R -0.35), \( p < 0.0001 \)

Furet JP, Kong LC et al  Diabetes 2010

Wilcoxon; * \( p < 0.05 \) D vs C/ND
**Concl (1) Gut microbiota differentially adapts after GBP**

*differential adaptation*

*links (Bact-Meta-Infl)*

*Food intake*

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**Metabolism**
- BMI, fat mass, Leptin, Insulin, etc
- *Bacteroides* (-neg)
- *E. coli* (-neg)
- *Bifido, Lacto* (+pos)

**Inflammation**
- CRPus, IL-6, etc
- *F. prausnitzii* (-neg)

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Model effect
Sequencing

?? ?? ?? ??
Microbiota-host interactions in obesity & related complications

Cani PD and Delzenne NM. Curr Opin Clin Nutr Metab Care 2007; 10:729–734
Conserved traits at various levels of integration:

Core microbiome

Criteria to qualify normobiosis/eubiosis

Microbiome specificities in diseases, towards:
identification of predictive biomarkers, new targets and
strategies for nutritional and/or therapeutic applications
in intestinal disorders