The intimate relationship of diet, microbiota and host-health

Multivariate Intertwined system

Diet: Composition & Intake

Microbiota: Composition & Function

Host: Energy balance & Immune Health
Increase in microbiota along digestive tract

Stomach
$10^0$-$10^4$ bacteria/ml

Small intestine
$10^4$-$10^8$ bacteria/ml

Colon
$10^{10}$-$10^{11}$ bacteria/g

Cells: 10-fold

Genes: > 100-fold
Microbiota composition analyses

“classical” culture based approaches

Highly skewed view

~ only 20-35 % cultured species

Culture independent molecular methods

16 S rRNA approaches
16 S rRNA based microbiota analyses

Microbiota composition analysis

Culture independent technology

16 S rRNA based species detection
✓ universally present in bacteria
✓ sequence based phylogenetic marker
✓ variable and conserved regions
✓ molecular phylogeny analyses

Community composition profiling and targeted population quantification
✓ barcoded pyrosequencing
✓ phylogenetic arrays
✓ qPCR
✓ FISH
✓ etc.
The human large intestine microbiota

- Molecular typing
- 1000's species identified to date
- 80 % in 3 major Phyla
  - Bacteroidetes
  - Firmicutes
  - Actinobacteria
- Dominated (~70 %) by uncultured
- Stable over time
- Resilient (antibiotic therapy)
- Subject-specific
- Composition association with health and disease
From species composition to function
Metagenomics of the human microbiome

THE PROLIFERATION OF HUMAN MICROBIOME PROJECTS

- Canadian Microbiome Initiative
  - $10 million
- MetaHIT (EU and China)
  - $31 million
- Human MetaGenome Consortium (Japan)
  - $5 million
- Microbes (France)
  - $3 million
- Meta-GUT (China)
  - $1.5 million
- Human Gastric Microbiome (Singapore)
  - $750,000
- Australian Urogenital Microbiome Consortium
  - $600,000

All figures are estimates of cost in US dollars


International Human Microbiome Consortium
The colon is famous
Unexplored small intestinal microbiota

Colon
- Good accessibility
- Well studied microbiota

Small intestine
- Poorly accessible
- Relatively unexplored

Who am I?
Main site for food digestion and absorption
First region where food meets microbiota
Major site for immunological perception

Peyer’s patches
Significance of small intestinal microbiota

Human small intestinal microbiota

Amenable to dietary modulation, and expected impact on host physiology
Sampling the human small intestine

Healthy subject
(Sampling with catheter)

Surgical removal of colon
Crohn's disease, Ulcerative colitis, and cancer

Ileostomy subject
(Non invasive sampling of luminal microbiota of the distal ileum)

Diversity of microbiota in small intestine
Molecular microbiota typing
Human Intestinal Tract Chip (HITChip)

Phylogenetic database

Phylogenetic chip

High resolution
High sensitivity

16S rRNA phylogeny

Intensity plots
Phytotype clustering
PCA
• Presence of species belonging to Bacilli and *Clostridium* cluster I
• Reduced abundance of species belonging to *Bacteroides, Clostridium* clusters IV & XIVa
Microbial composition of the small intestine microbiota

Ileostoma effluent microbiota
Prominent presence of:

- *Streptococcus* spp.
- *Veillonella* spp.
- *Clostridia* spp.
- *Escherichia* spp.

"Common core" microbiota detected in ileal effluent of five subjects with an ileostoma

- *Clostridium* cluster I
- *Clostridium difficile* et rel.
- *Enterococcus*
- *Oxalobacter formigenes* et rel.
- *Streptococcus bovis* et rel.
- *Streptococcus intermedius* et rel.
- *Streptococcus mitis* et rel.
- *Veillonella*
Ileostoma effluent as a model for proximal small intestine microbiota

Healthy subject (Sampling with catheter)

Co-clustering of effluent, jejunal, and ileal communities; distinct from terminal ileum and fecal communities

Zoetendal et al. 2012 ISME J
From composition to function
Human Small Intestine Metagenome

<table>
<thead>
<tr>
<th>Metagenome (4 subjects)</th>
<th>Metatranscriptome (4 subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Sequencing 7.5 Gbp</td>
<td>• mRNA enriched</td>
</tr>
<tr>
<td>• ~ 200,000 non-redundant genes</td>
<td>• Random-primed cDNA library</td>
</tr>
<tr>
<td>• Primary function annotation</td>
<td>• Sequencing 50 M, Illumina HiSEQ</td>
</tr>
<tr>
<td>• Small intestine gene catalogue</td>
<td>• Profiling (comparative)</td>
</tr>
<tr>
<td></td>
<td>– Function &amp; phylogeny</td>
</tr>
</tbody>
</table>

Zoetendal et al. 2012, ISME J
Metabolic projection of metatranscriptomes

Subject 1, 2 & 4

Streptococcus
Clostridium
Veillonella
Syntrophic relations in the microbiome

Task division between members

Focus on CHO-import and conversion

> Product lactate

Focus on secondary lactate metabolism

> Lactate to acetate / propionate
Prototype ecosystem model for the small intestine microbiota

**Ecosystem Model**

1. composition
2. functionality
3. task division & dependencies

**Impact of:**

- Dietary modulation of community structure?
- Dietary modulation of fermentative output?
lactobacilli in the small intestine?
Prominent community impact of probiotic intervention

- *Lactobacillus* species are not dominant in small intestine communities
- Probiotic intervention can provide temporal community shift
- Prominent presence of *Lactobacillus*
- Impact on mucosal biology?

Probiotic product administration
Single serving ~ $10^9$ cells
Probiotic modulation of the small intestine microbiota

Probiotics: “Live microorganisms that when administered in adequate amounts confer a beneficial health effect on the host”

Dominated by lactobacilli & bifidobacteria

In some cases “anecdotal” documentation of health benefit
Limited clarity for some health benefits in meta-analyses
Probiotic interactions

Species & strain specificity?

Molecular mode of action?

Nutrigenomic approach to unravel molecular mode of probiotic activity.
Nutrigenomics

“Nutrigenomics is the study of the effects of foods and food constituents on gene expression. It evaluates how our DNA is transcribed into mRNA (and translated into proteins) and provides a basis for understanding the biological activity of food components.”

Analysis of whole genome (human) response to probiotic intervention……
Nutrigenomic analysis of probiotic effects in humans

6 HOUR ORAL INTAKE PROBIOTIC STUDY
- Healthy human volunteers, all males (n=8)
- 4 interventions, randomized, placebo-controlled double-blind, cross-over design.
- 2 week wash-out

6 hour intake regime (every 30 min a “probiotic” drink)
- *L. acidophilus* Lafti-L10 (DSM, the Netherlands), *L. casei* CRL-431 (Chr. Hansen, Denmark), and *L. rhamnosus* GG (Valio, Finland)
- All at ~ 2 to 5*10⁹ dosage
- Reconstituted freeze dried bacterial preparations in maltodextrin suspension (placebo = no bacteria)

Van Baarlen et al. PNAS, 2009; 2011
Detection of specific responses per probiotic?

- Significant transcription response (p < 0.05)
- **300-750** genes affected by probiotic consumption
- Majority **1.3-2.5** fold-change; some major responses > **10-20** fold change
- **Unidirectional** (max. 1 directional outlier tolerated)
- qRT-PCR validated (25 genes)

Van Baarlen et al. PNAS, 2009; 2011
Data interpretation
an integrated view of mucosal response

Differentially expressed genes

RESPONSE

PATHWAYS

NETWORKS

COMPARISON

Cytoscape
An Open Source Platform for Network Analysis and Visualization

INGENIUTY SYSTEMS

BiblioSphere Pathway Edition

VISUALISATION & RECONSTRUCTION

GSEA
Gene Set Enrichment Analysis

nutrigenomics consortium
Distinct responses to lactobacilli
Gene Set Enrichment Analysis

- L. acidophilus
- L. casei
- L. rhamnosus
Distinct responses to lactobacilli
Most dominant pathways

- **L. acidophilus**
  - Immune response pathways
  - Hormonal signaling

- **L. casei**
  - Immune balance Th1/Th2
  - Proliferation
  - Metabolism & ion homeostasis

- **L. rhamnosus**
  - Wound healing
  - Angiogenesis
  - Ion homeostasis

Van Baarlen et al. PNAS, 2009; 2011
Connecting to clinical observations
Immune regulatory networks affected by *L. acidophilus*

- Ingenuity pathway reconstruction
- Key-signaling pathways IL-10, IL-8 and NF-κB, affected by *L. acidophilus*
- Modulation of innate & adaptive immune response
  - innate immunity stimulation
  - stimulation of Th1 responses

**Innate immunity stimulation**
Mouse and rat
Goldin and Gorbach 1980; Gilliland 1989; Fernandes and Shahani 1990; Gill et al 2000

**Immune stimulation (Th1)**
enhanced Th1 signaling, human & mouse
Hypothesis generating models that aid to explain (predict) clinical effects

Van Baarlen et al. PNAS, 2011
Data interpretation

An integrated view of tissue response

L. acidophilus
L. casei
L. rhamnosus
Interpersonal variation exceeds intervention response

Pearson distance matrix:
- Intervention ~ 0.005 – 0.010
- Individual ~ 0.012 – 0.025

The band-width of health

Homeostasis / health

- Stable & individual baseline molecular profile
- Consistent modulation by probiotic intervention
- Cross-over design required!
- Physiological consequences?
  - can all individuals perceive this effect?
  - explain “non-responders” in probiotic trials?
- Basis for individuality?
  - genotype / epigenetic imprinting
  - dietary habits / lifestyle
  - endogenous microbiota
- Basis for subject stratification & response prediction?
Conclusions

- Metagenomics
  - Small intestine community
  - Composition and activity, subject to dietary modulation
  - Metabolic task division (syntrophy)
  - Major community impact of dietary lactobacilli consumption

- Nutrigenomics
  - Conserved & species specific response to probiotics
  - Molecular responses give mode of action support to clinical outcomes
  - Prediction of novel probiotic application?
  - Human individuality in relation to nutrition impact?

- Metagenomics and Nutrigenomics offer novel strategies for functional food design & mechanism based health claims for stratified subpopulations
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