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Introduction to the intestinal microbiota

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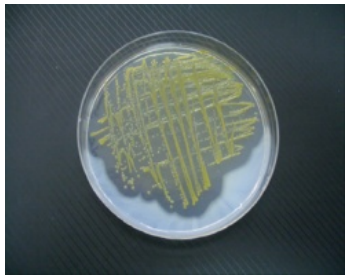
Effects of gut microbiota on host health

Responsible for **Integrity** and **Function** of the GI Tract:

- **Barrier effect** **(Competition for attachment sites and available nutrients, inhibition of pathogens growth** *(alteration of intraluminal pH, reduction of redox potential, bacteriocins production)*
- **Stimulation of the immune system** **(Induction of the maturation of gut-associated lymphoid system; stimulation of specific systematic and local immune responses)**
- **Metabolic/Trophic function** **(Harvest calories from complex polysaccharides through colonic production of short chain fatty acids; SCFA affects lipid storage and metabolism; vitamin synthesis)**
- **Drug/Toxin metabolism** (Xenobiotics biotransformation, carcinogen synthesis and activation)

Methods for analysis of intestinal microbiota

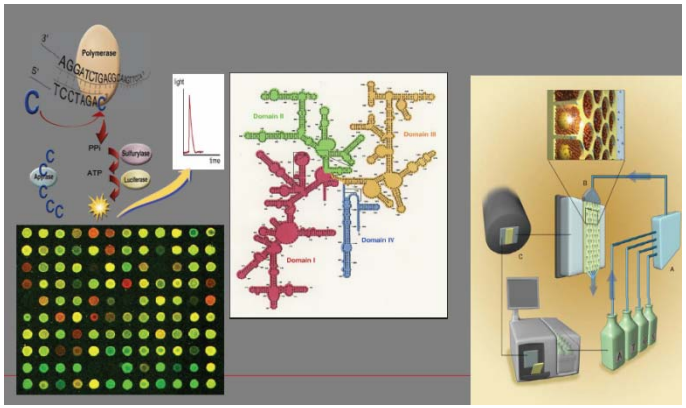
- **Culture-based methods allow to recover <30% of total microscopic counts**



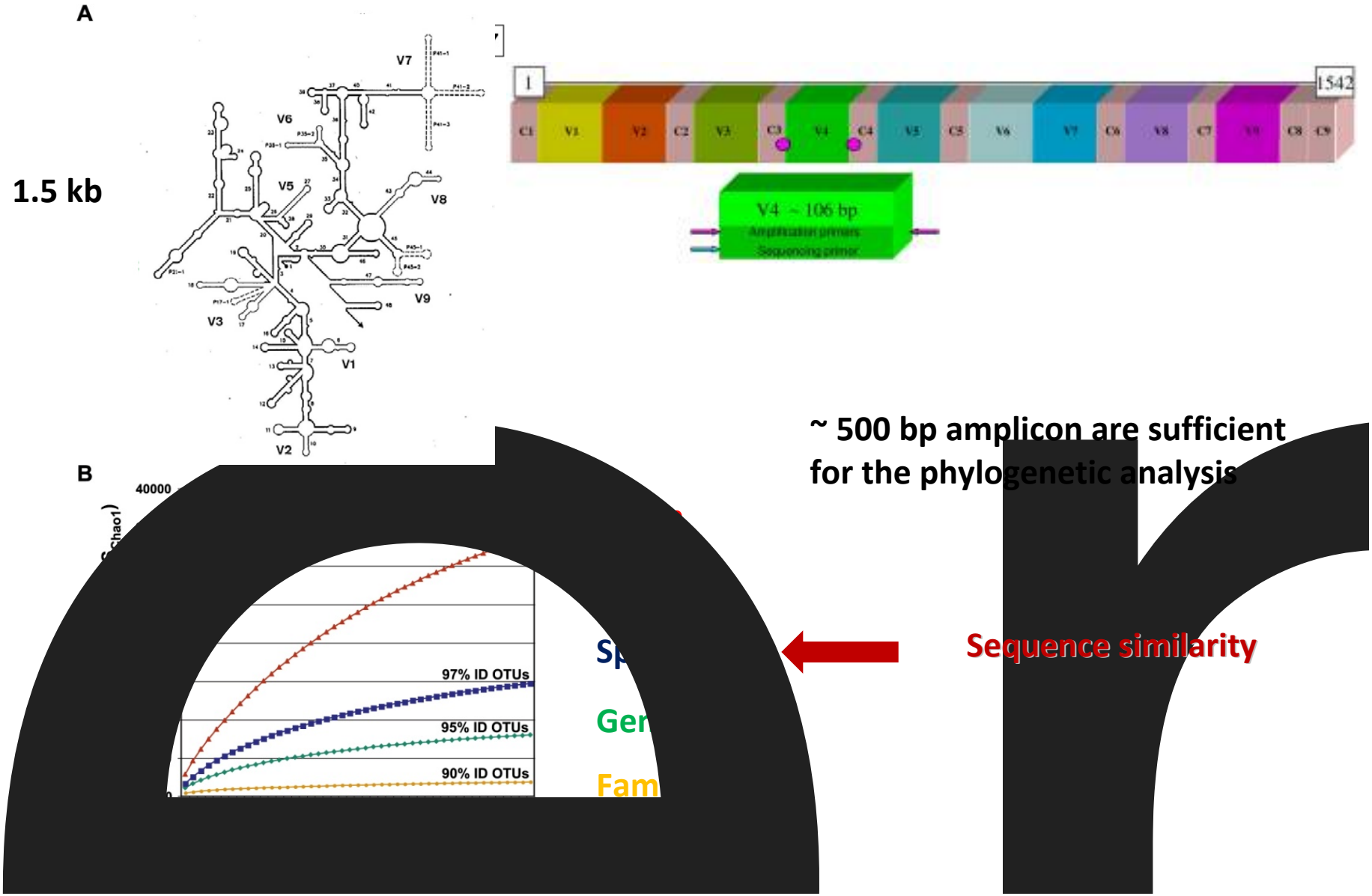
MOSTLY UNCULTURED!

anaerobic cultivation technologies
small size of some groups
nutritional requirements

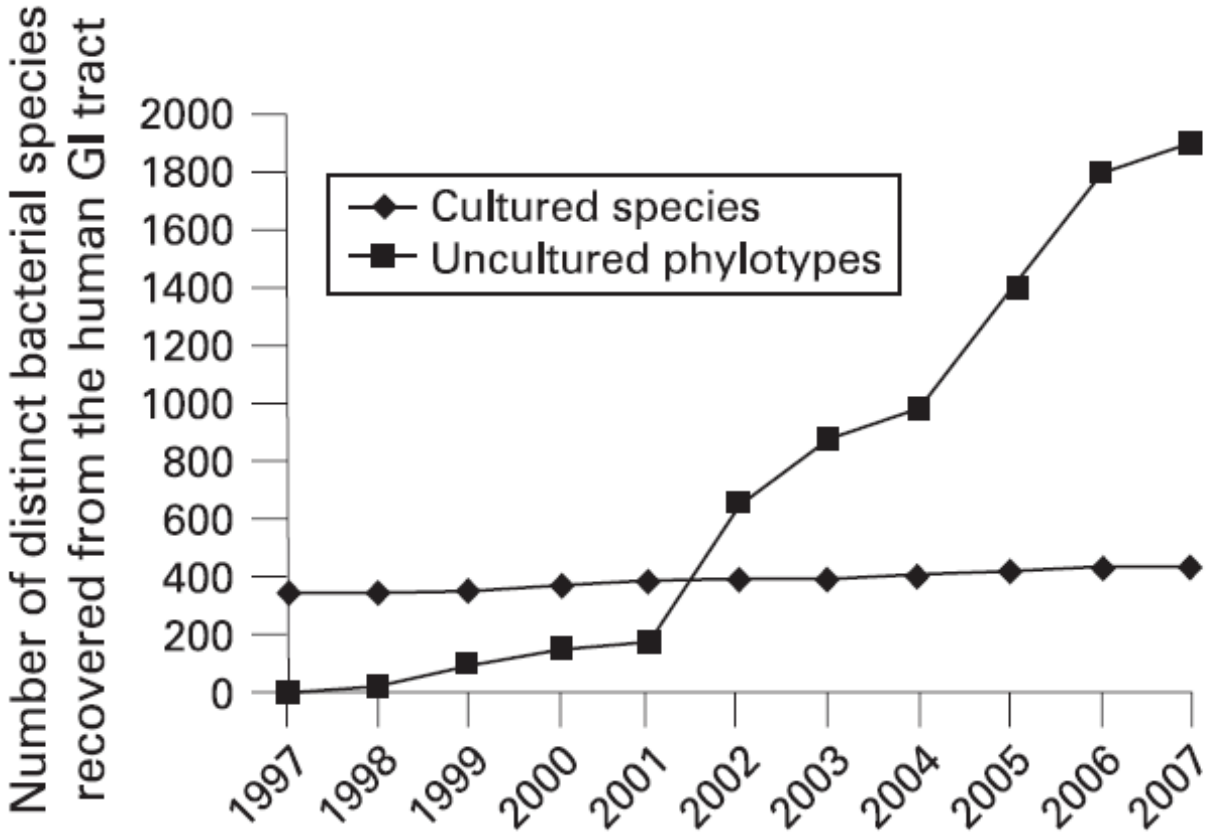
- **Culture-independent molecular techniques: PCR based analysis of 16S rDNA**



16S bacterial Small Subunit rRNA gene-based survey of the gut microbiota



Gastrointestinal phylotypes detected with culture-dependent and culture-independent approaches



Zoetendal et al., Gut 57, 1605-1615, 2008

Composition and metabolic function of GUT microbiota

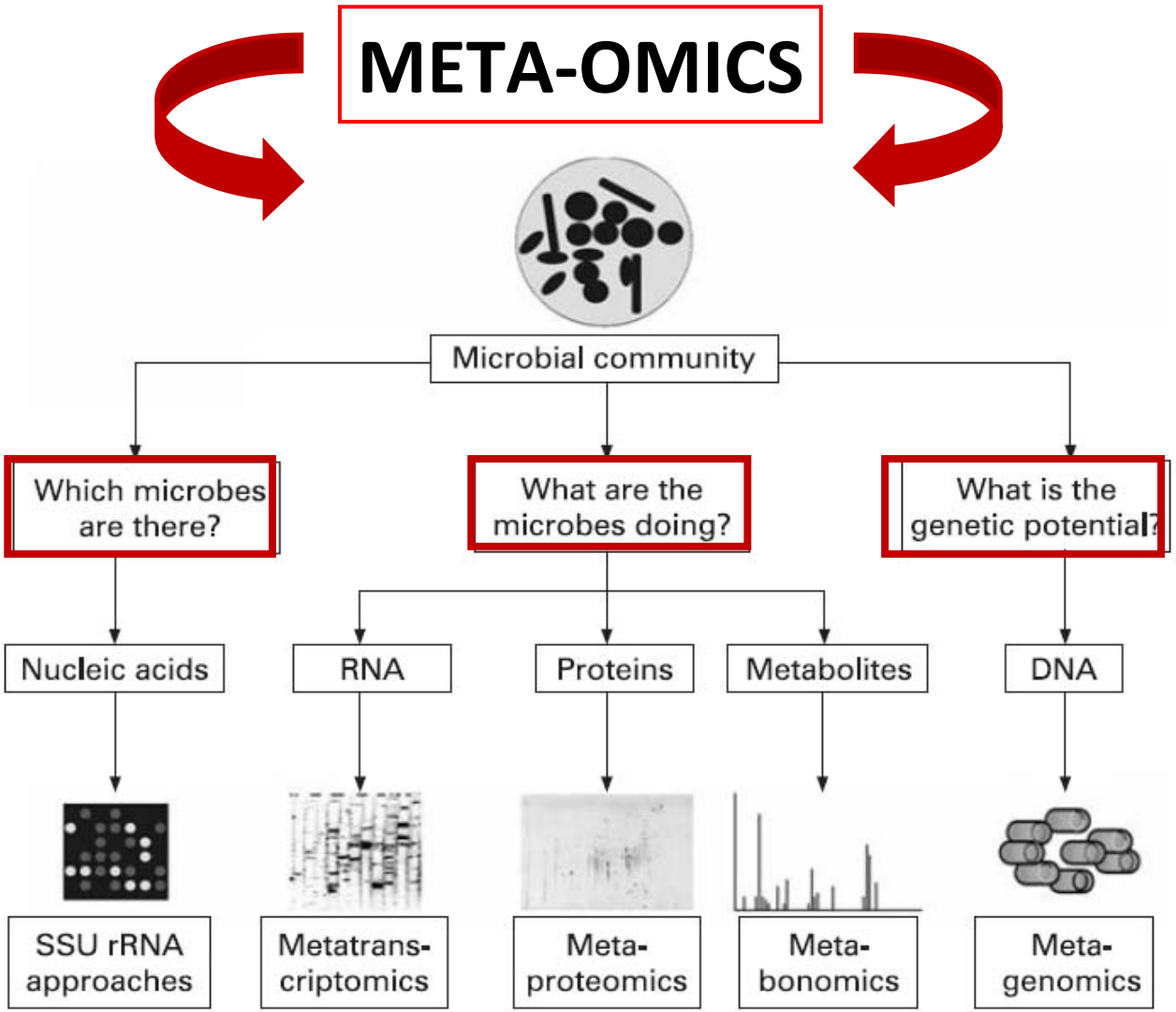


Figure 3 Schematic representation of the metagenomics and other community-based "omics" approaches. SSU rRNA, small subunit ribosomal RNA.

Metagenomics

profiling intestinal microbiota at high resolution by studying the collective set of genomes

Challenges:

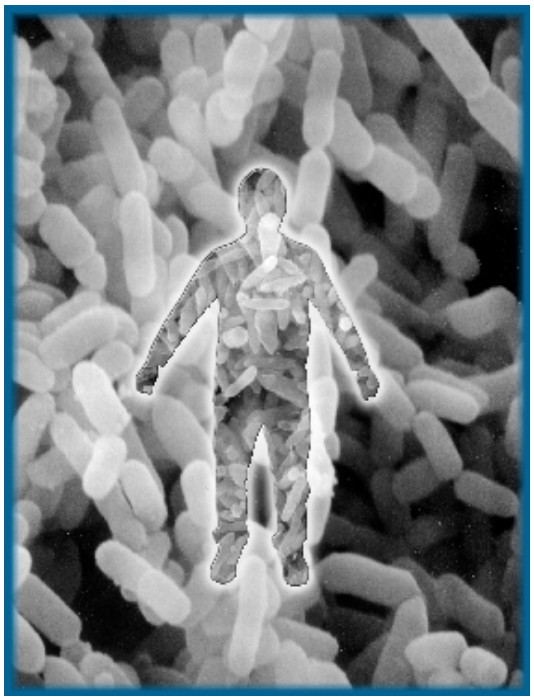
- **Phylogenetic characterization:** obtaining accurate microbial identification for thousands of gut microbiota species in a reasonable time and for a reasonable cost

- **Functional metagenomics:** to identify and annotate diverse arrays of microbial genes that encode many different biochemical and metabolic functions of the gut microbiota

The human distal gut harbors an immense microbial ecosystem

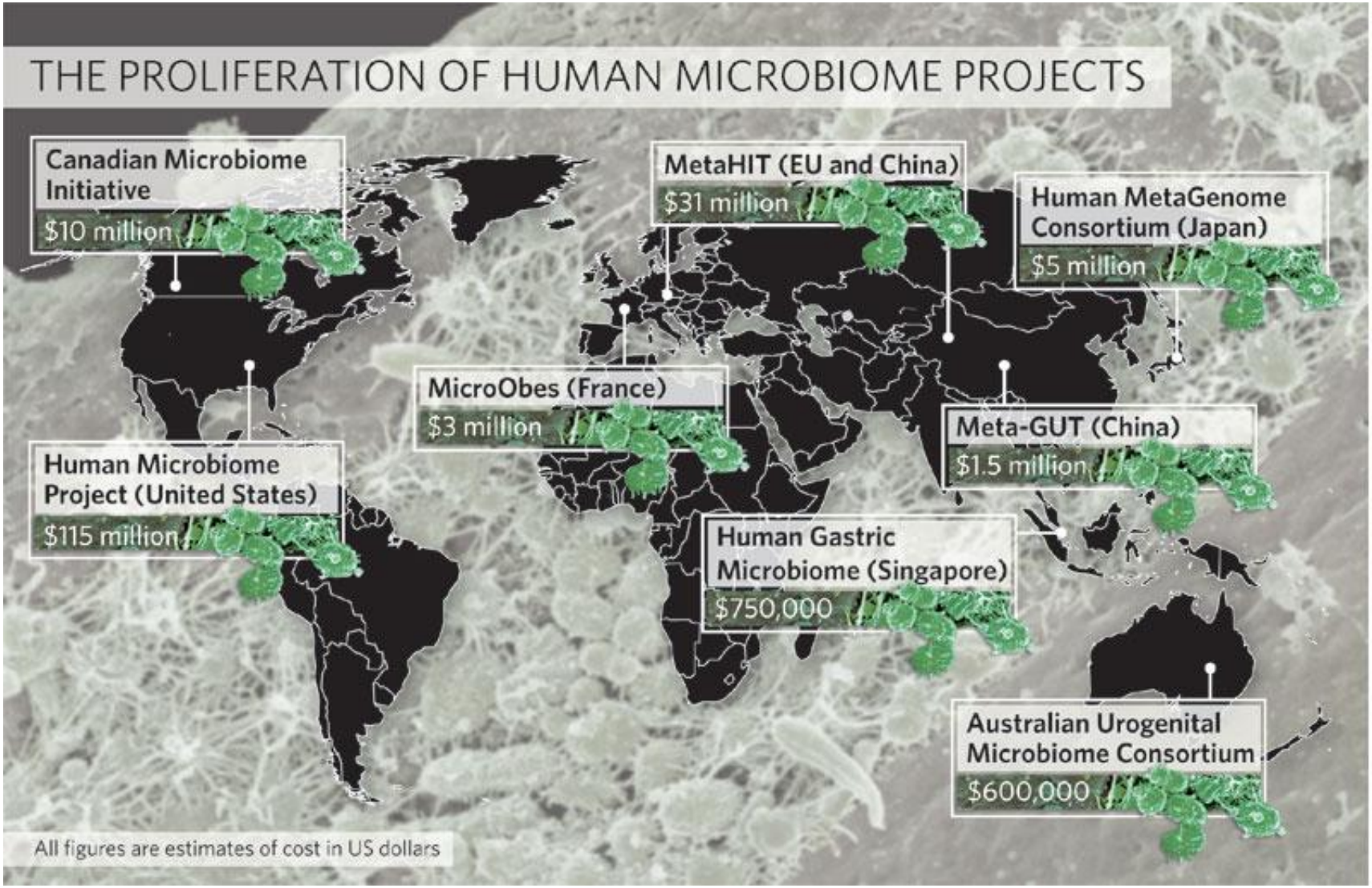
- 10^{14} microbial cells (anaerobic and aerobic bacteria, yeast, virus)
- 150 times more genes than human genome
- wide range of metabolic functions we did not evolved on our own

We are "metaorganisms"
result of millennia of co-evolution
with an incredible number of symbiont
microbial cells living in our body



Human microbiome

Collection of microorganisms living in and on the human body



The human intestinal microbiota has been characterized by metagenomics from a limited set of individuals (~1000/6.7 billion)

62% of human gut bacteria identified were previuosly unknown

80% of gut bacteria identified were non-cultivable

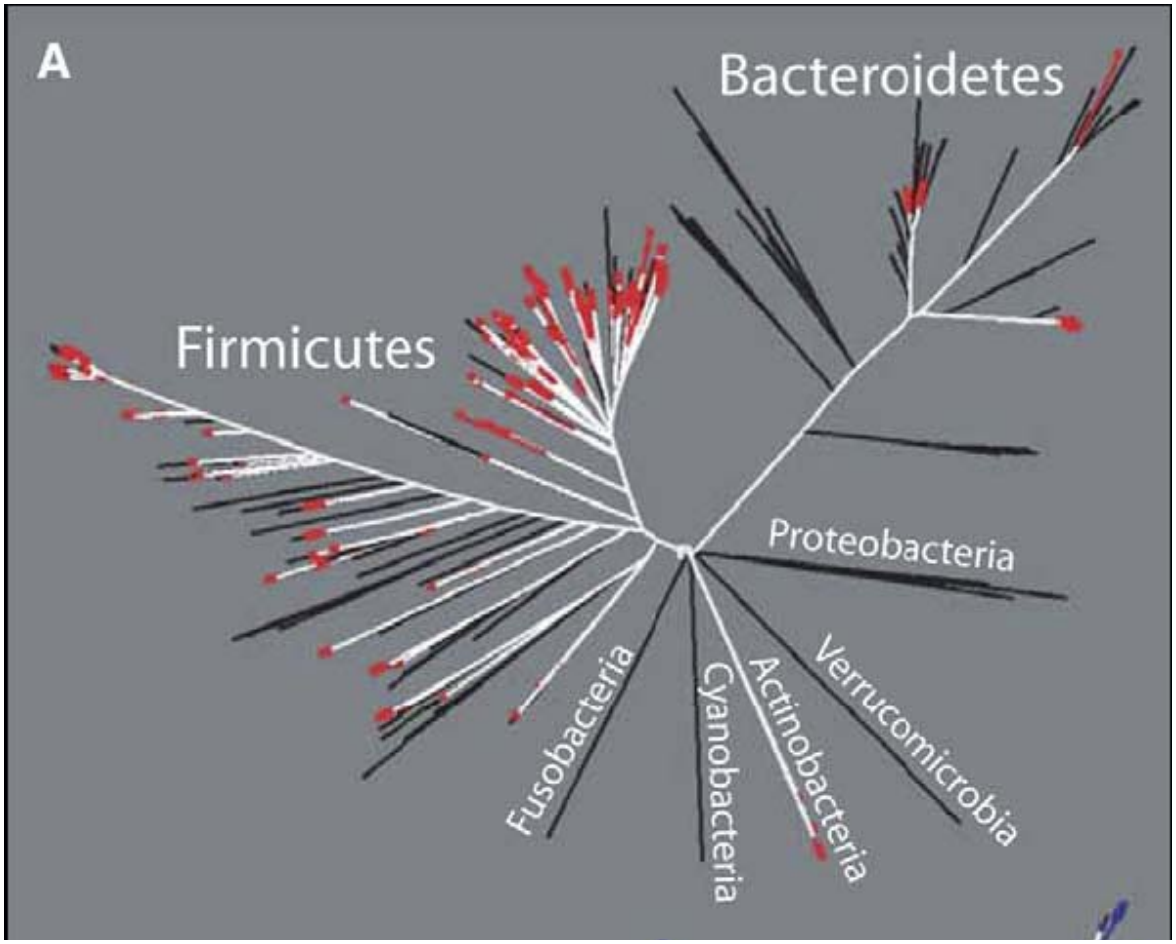
We are still at the beginning of describing the GIT microbial diversity: functions and identity of most gut microbes remain unknown

(Eckburg *et al.,* Science 2005; Ley *et al.,* Cell, 2006; Turnbaught *et al.*, Nature, 2007; Turnbaught *et al.*, Nature, 2009; Qin et al., Nature 2010; Willing *et al*., Gastroenterology, 2010; Larsen et al., PlosOne 2010)

Metagenomics
phylogenetic characterization of the human gut microbiota

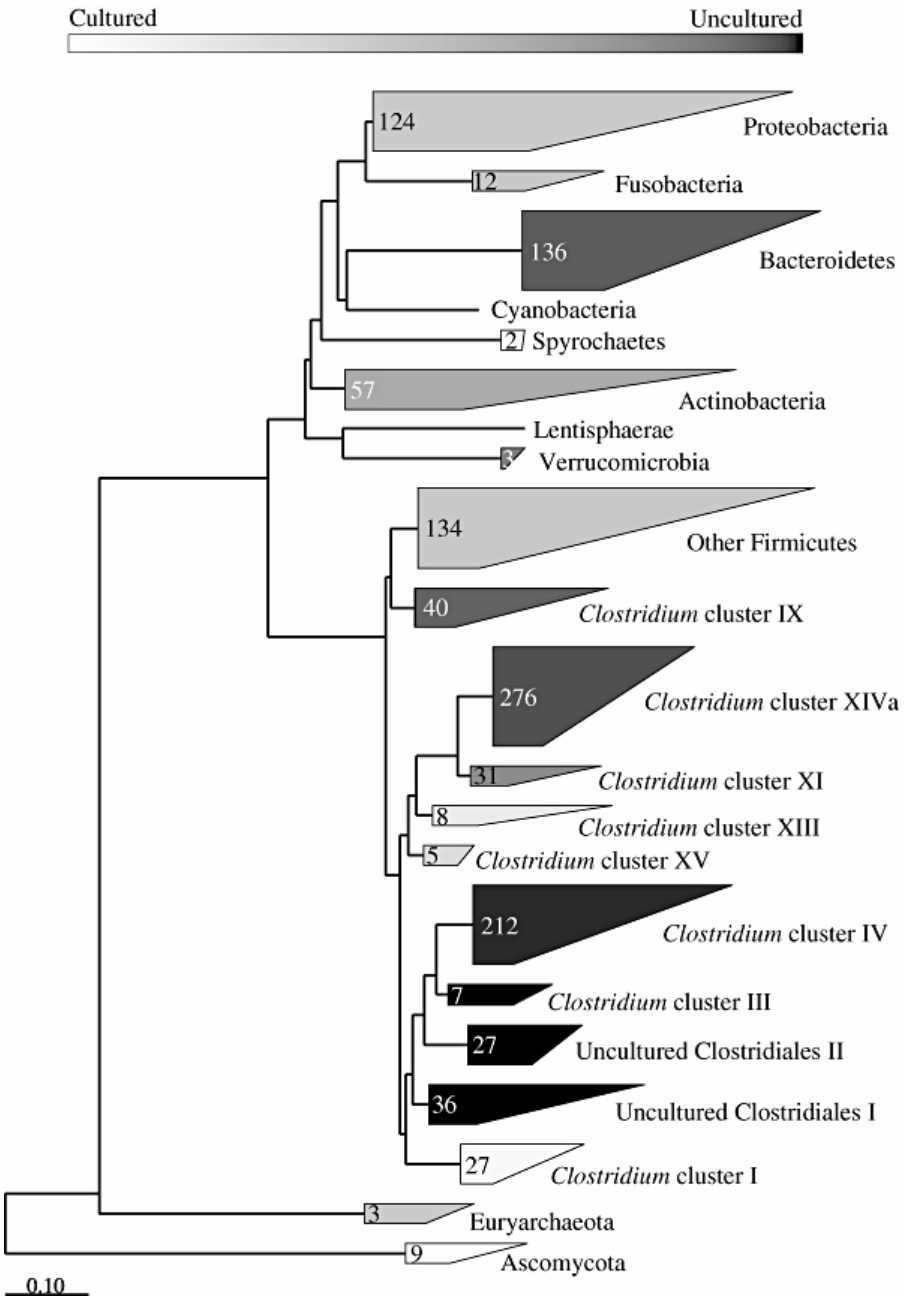
> 90% **2 bacterial dominant divisions** (*Bacteroidetes, Firmicutes);* < 10% **5 sub-dominant divisions** (*Actinobacteria, Proteobacteria, Fusobacteria, Cyanobacteria* and *Verrucomicrobia*)

A single archaeon *Methanobrevibacter smithii*



Bacteroidetes (*Bacteroides*, *Prevotella*), Firmicutes (*Clostridium, Bacilli, Eubacterium, Mollicutes, Lactobacillus*, *Streptococcus*) Actinobacteria (*Bifidobacterium, Micrococcus*), Cyanobacteria, Fusobacteria (*Fusobacterium*), Proteobacteria (*Enterobacteriaceae*, *Pseudomonas*, *Xanthomonas*, *Neisseria*, *Campylobacter*, *Desulfovibrio*), Verrucomicrobia

Composition of the human gut microbiota



Total diversity of a healthy adult gut ecosystem:

1800 genera (≥90% sequence identity)

16,000 phylotypes at the species level (≥97% sequence identity)

(Zoetendal *et al*, 2008; Peterson *et al*, 2008; Petrosino *et al* , 2009)

90-99%

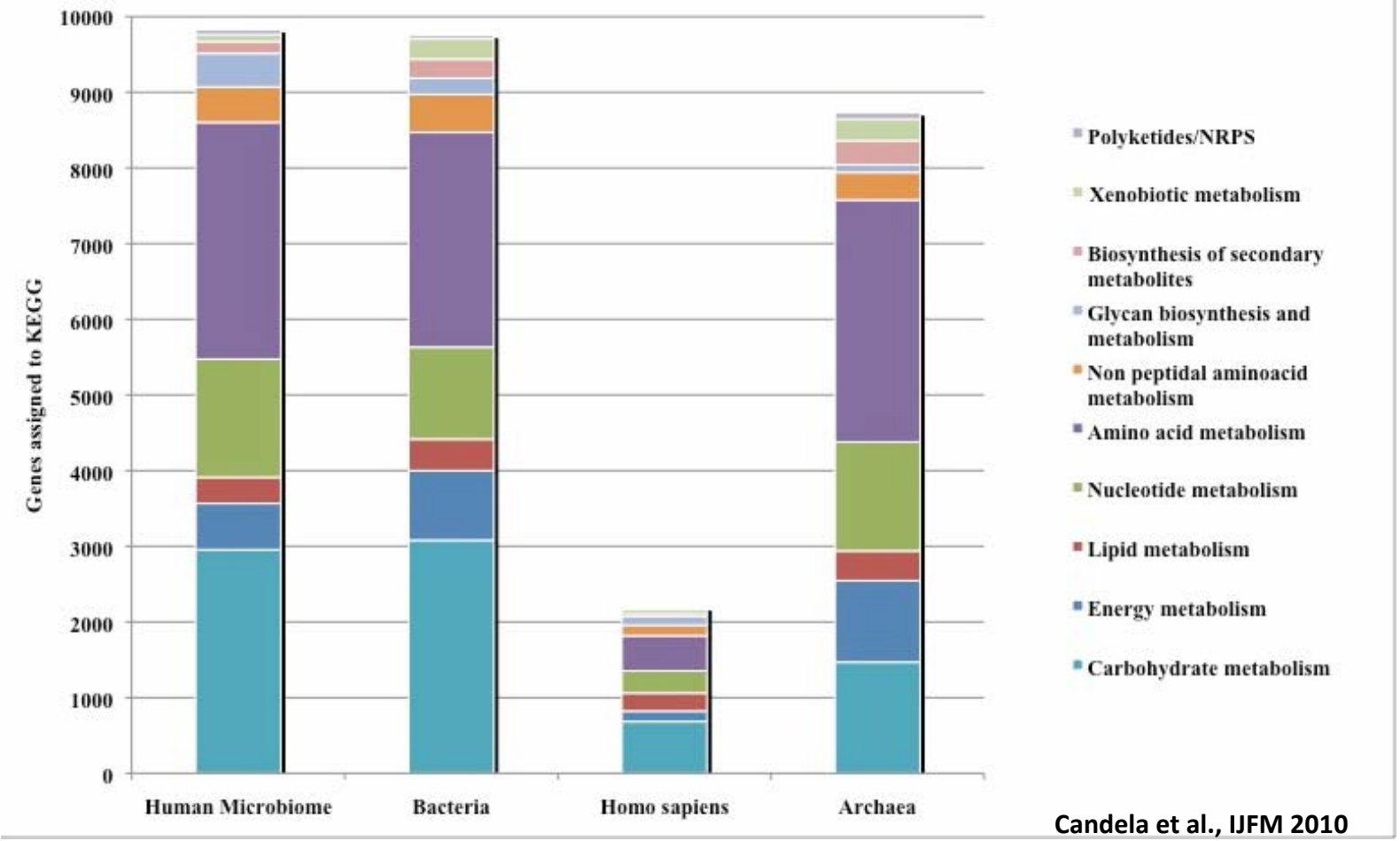
Bacteroidetes and Firmicutes

50-80% Firmicutes,
dominant *Clostridium* clusters IV and XIVa

Actinobacteria (3-15%) **Proteobacteria** (1-20%) **Verrucomicrobia** (0.1%)

(Eckburg *et al*, 2005; Ley *et al*, 2006; Turnbaugh *et al*, 2006; Frank *et al*, 2007; Andersson *et al*, 2008; Dethlefsen *et al*, 2008; Tap *et al*, 2009)

Metagenomics analysis: gene functional categories



Candela et al., IJFM 2010

Metabolic activity of the human gut microbiota corresponds to an extra organ equivalent to the liver

Significance of the genetic complement of the intestinal microbiota

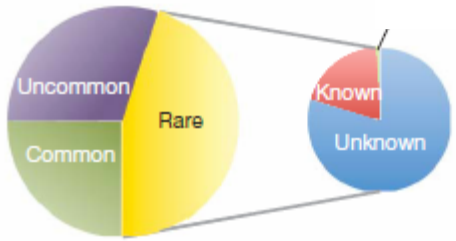
70% more novel sequences

minimal gut bacterial genome
contains required genes for gut bacteria to compete in the gut (i.e. genes encoding essential housekeeping, cell–cell adhesion proteins, enzymes required for degradation of complex sugars)

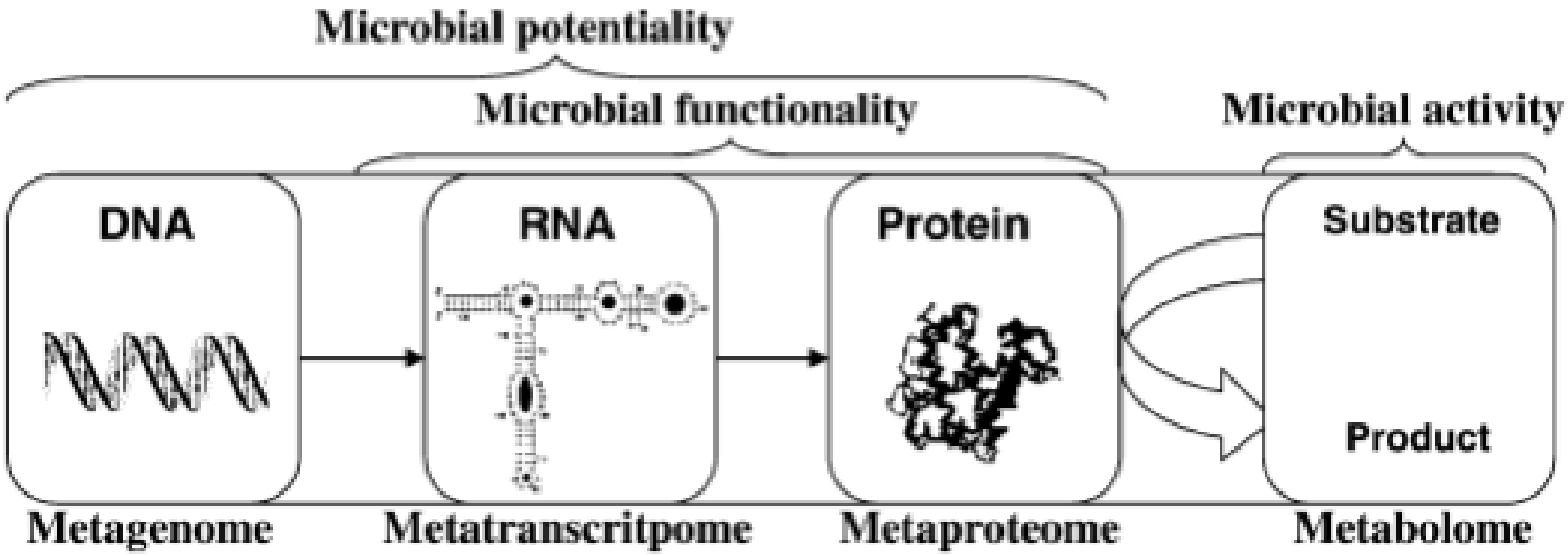
By characterizing the minimal gut bacterial genome, we hope to learn how selected bacteria became successful gut colonizers. This knowledge could be used to modulate the gut microbiota for therapeutic purposes.

minimal gut metagenome
encodes conserved functions for the complete gut community (it represents a set of core functions shared in all healthy hosts).

Of the sequences of the minimal gut metagenome, **only a few could be assigned characterized functions** (i.e. sugar fermentation, biosynthesis of SCFAs, amino acids, and vitamins, all of which are beneficial for the host).



Meta levels in the ecology of intestinal microbiota



Profiling microbiota at high resolution

Detection of changes in gene expression over time or under various physiological/environmental conditions

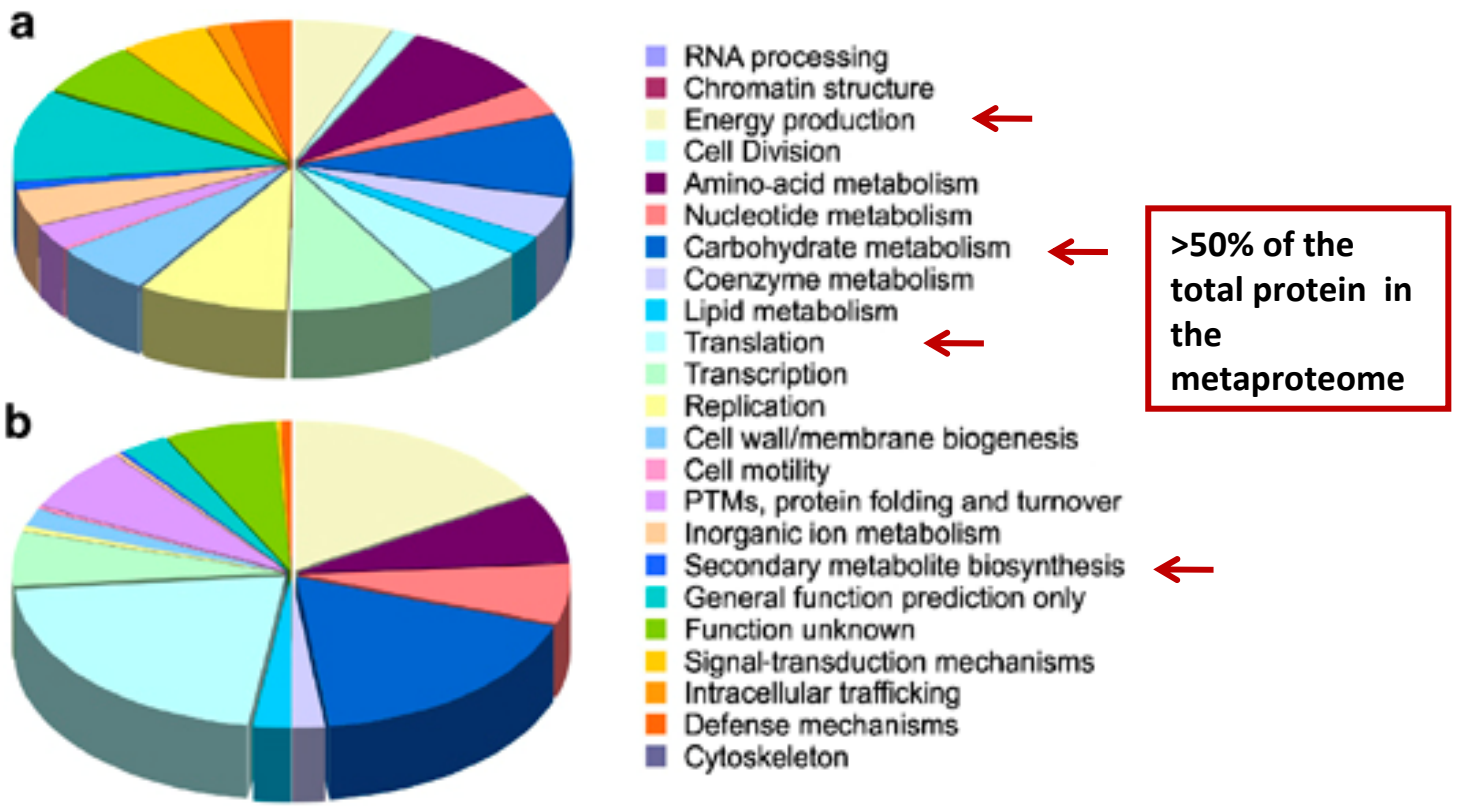
Linking community structure with function and genotypes with phylotypes.

Large-scale characterization of the entire protein complement expressed by intestinal microbiota

•Tracking new functional genes and metabolic pathways
•Identifying proteins preferentially associated with specific stresses
•Identifying functional bioindicators

Characterization of metabolic profiles associated to the microbiota

Metaproteomics of the human distal gut microbiota



Comparison of average clusters of orthologous group (COG) categories for available human metagenomes and metaproteomes. (**a**) Average COG categories of the two *metagenomes* from the gut microbiota of two individuals; (**b**) compared to average COG categories of the *metaproteomes* from the gut microbiota of two individuals

Verberkmoes et al., ISME J 2009

Open questions

- functions of most microbial inhabitants
- which of the many hundreds of species are of “key” importance in host health
- molecular host-microbiome interactions that influence host metabolic pathways
- identification of lost core functions to be associated with diseases

deciphering the genetic information contained in the microbiome by whole-genome shotgun sequencing

THANKS FOR YOUR ATTENTION!!!