Replication rate of bacteria in complex ecosystems

- Culture-independent methods strongly increased knowledge on microbial ecology complexity and metabolic potential
- To understand their individual contributions to the community (and potential benefit or risk), key to define:
  - ❑ are they live or dead?
  - ❑ do they replicate (multiply, grow)?

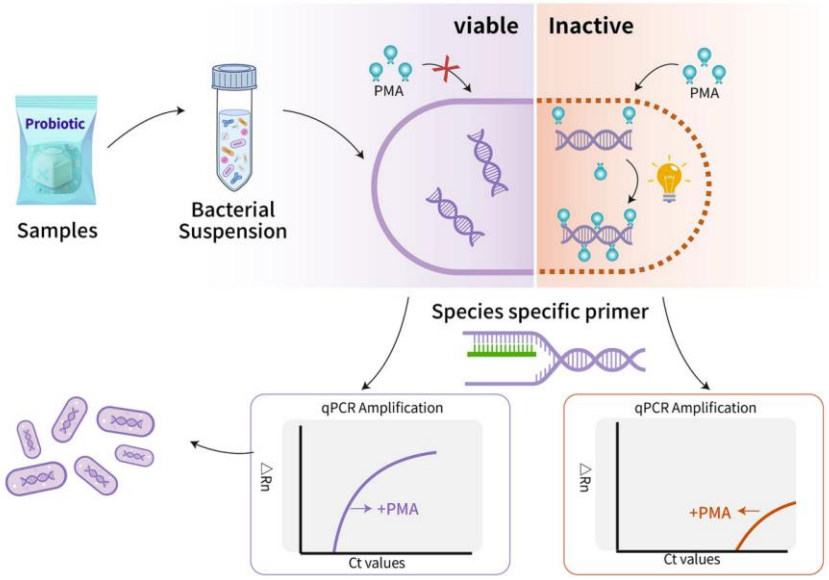
Distinguish live from dead

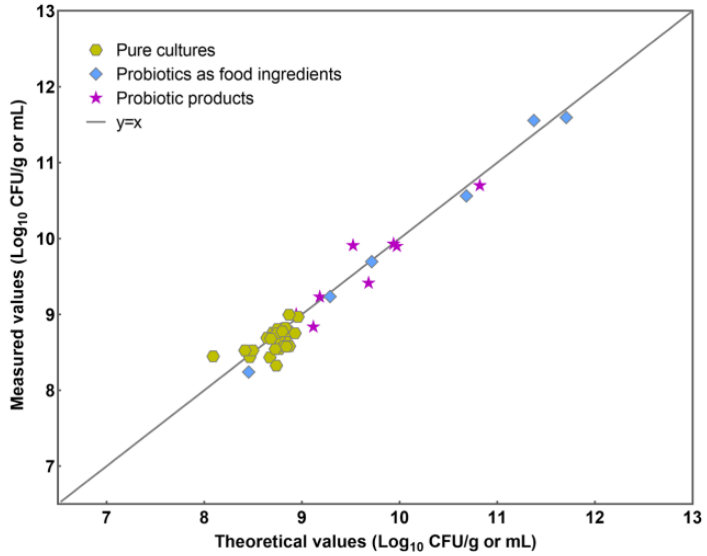
- Classical microbiology (plating, counting, Flow cytometry)
- Molecular methods based on DNA or RNA (culture independent)
- Performances are highly dependent upon matrix (water / liquid versus solid), quantities and complexities

PMA and derivatives

Principle: dye that penetrates dead (membrane damaged) cells, binds DNA and upon light exposure, makes it non amplifiable by DNA/Taq Pol
Drawbacks: requires standard curve for quantification (e.g. CFU equivalent), and optimal PMA conditions may vary for different strains (no universal conditions)

Example for probiotics and using qPCR but could be applied to other sample types and NGS used as readout





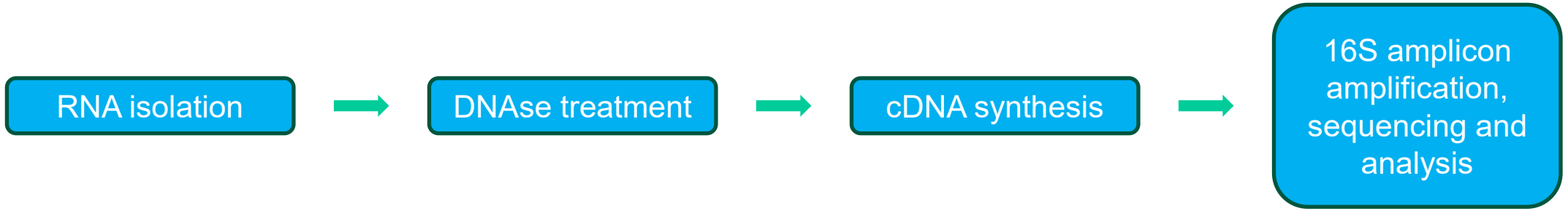
Guo et al, Frontiers in Microbiology, 2024

RNA-based measures

Principle: live cells (metabolically active or dormant) have RNA intact and hence more abundant, as opposed to degraded RNA in dead cells

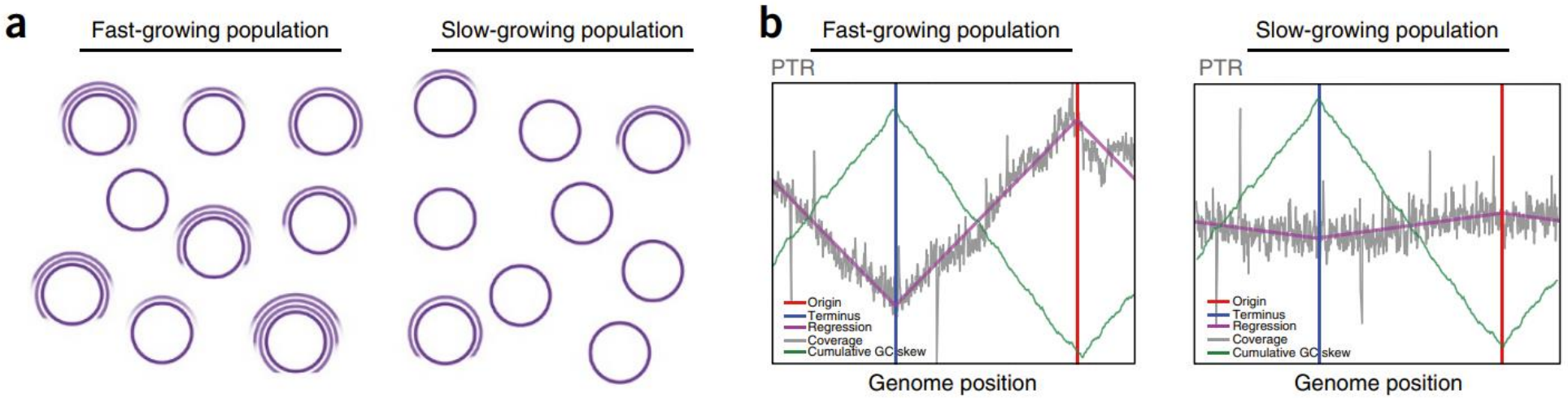
rRNA analysis most common

Protocol:



Analysis of bacterial replication

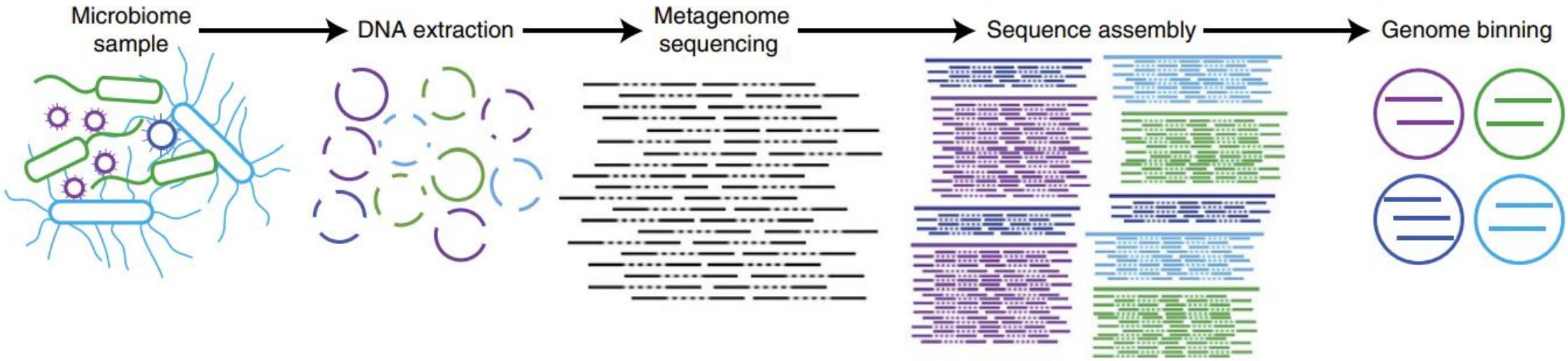
- Bacterial genome replication proceeds bi-directionally from a single origin of replication
- In an unsynchronized population of growing bacteria, cells contain genomes that are replicated to different extents, resulting in a **gradual reduction in the average genome copy number from the origin to the terminus** of replication



- Brown, Nature Biotechnology 2016

Analysis of bacterial replication

- This can be detected by measuring changes in sequencing coverage across complete genomes
- Requires “relatively” good level of assembled genomes of the bacteria present



- Brown, Nature Biotechnology 2016

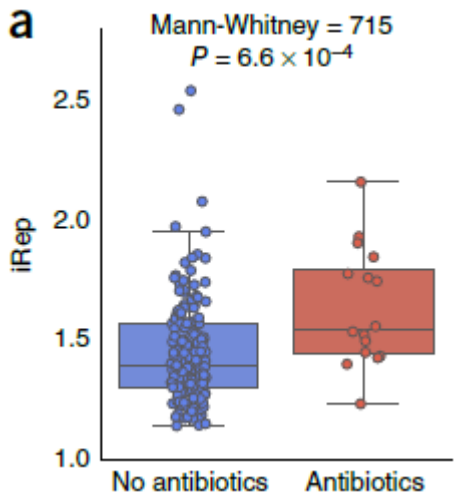
Method

- Use of the total change in coverage across all genome fragments
- Mapping metagenome sequencing reads to the collection of assembled sequences that represent a draft genome (not strictly linked to completion of data base used for mapping) => **accurate for complete or draft genomes**
- Extreme high- and low-coverage windows are excluded (more than 8 x Δ to the median), as well known to correlate with highly conserved regions, strain variation, or integrated phages

Example of findings and outlook

Elevated replication rates are associated with antibiotic administration

samples collected during or within 5 d after antibiotic administration and samples from other time points



Establishment of models that can accurately predict microbial community dynamics and functions under changing environmental conditions

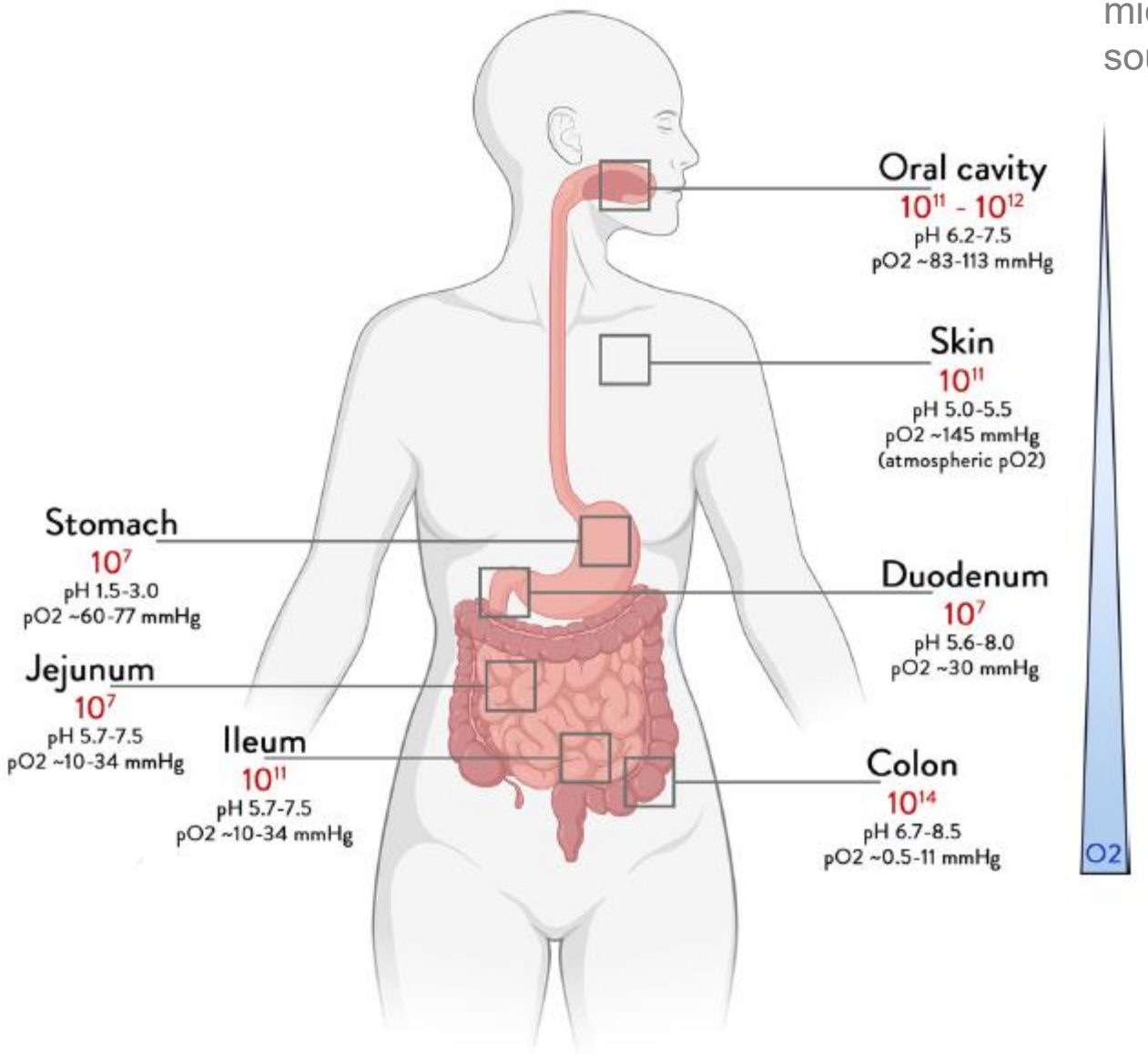
Potential to improve our understanding of relationships between bacterial functions and biogeochemical processes or health and disease

Challenges with low-biomass microbiomes

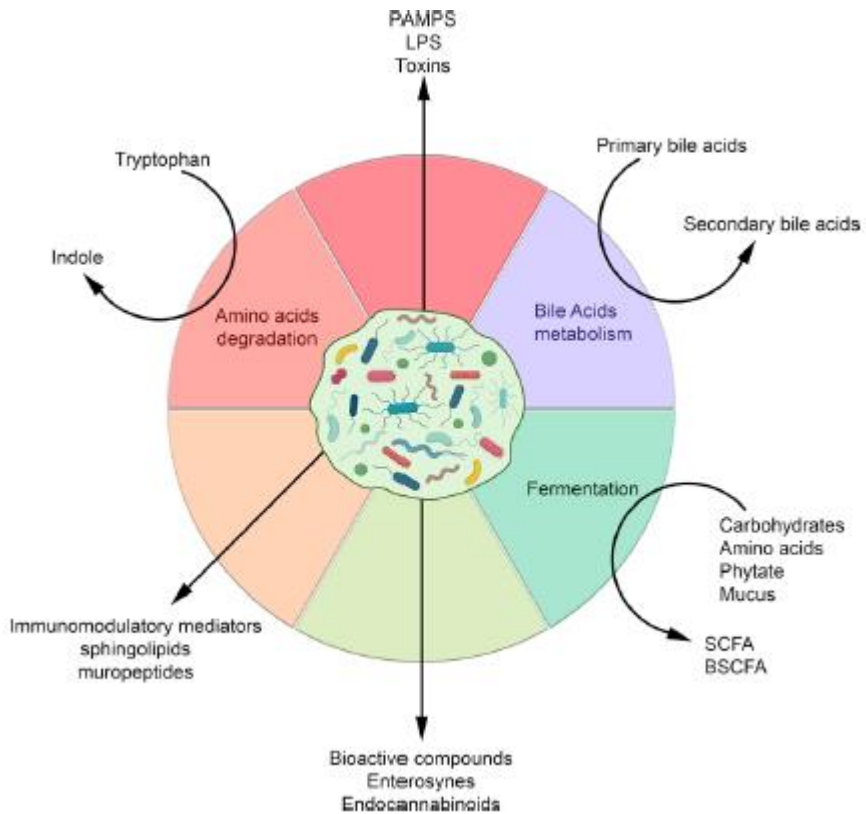
Skin, lungs, reproductive organs, bile ducts

- Low to extremely low levels of micro-organisms
- Challenging to distinguish real microbial signatures from noise or contaminations
- Contaminating DNA can originate from:
  - DNA extraction in the lab
  - PCR and library prep enzymes (themselves produced by overexpression in bacteria)
  - Appropriate positive and negative controls, and follow up validation required

Total abundance of bacteria according to the different body sites



Molecules and metabolites produced by the gut microbiota according to the nutrients or metabolic source and their derived compounds



de Vos WM, et al. Gut 2022

Evolution of the microbiome from birth to first years

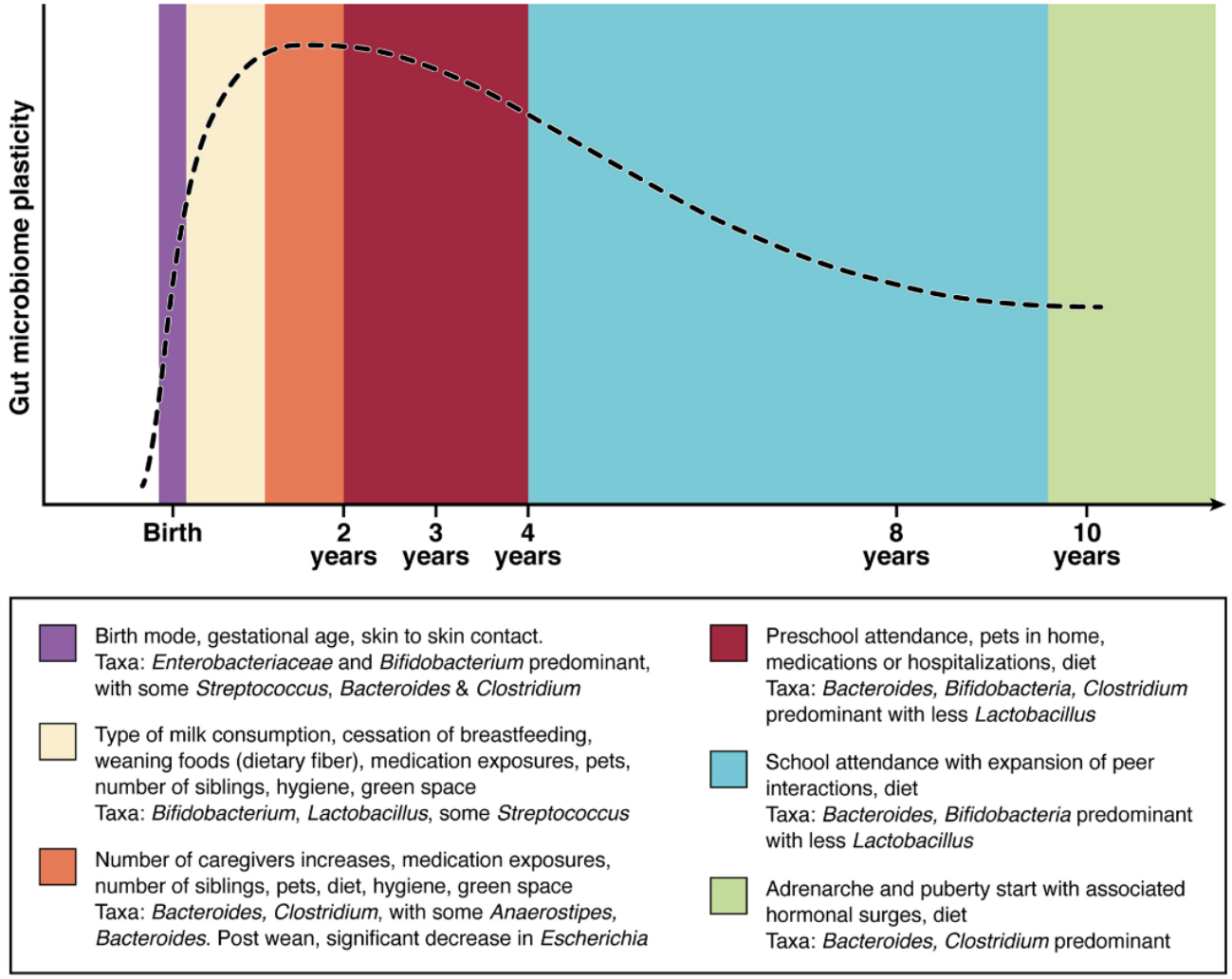
Clostridia, Enterobacteria, and Streptococci were observed in the infant intestines in the first 2 days after birth

Bifidobacterium longum appear and become dominant between days 4th and 7th

For digestion of HMOs

Contributes to development of immunity

Before weaning, the relative abundance of Bacteroides gradually increases to compete for Bifidobacterium in the infant intestine



Ronan et al, Gastroenterology, 2021

Case study: identification of a new clade of *Bifido. longum*

CellPress

Cell

Article

A distinct clade of *Bifidobacterium longum* in the gut of Bangladeshi children thrives during weaning

Tommi Vatanen,[1,2,3,11] Qi Yan Ang,[1,11] Léa Siegwald,[4,11] Shafiqul Alam Sarker,[5] Caroline I. Le Roy,[4] Stéphane Duboux,[6] Omar Delannoy-Bruno,[4] Catherine Ngom-Bru,[7] Claire L. Boulangé,[4] Martin Stražar,[1] Julian Avila-Pacheco,[1] Amy Deik,[1] Kerry Pierce,[1] Kevin Bullock,[1] Courtney Dennis,[1] Shamima Sultana,[5] Sharika Sayed,[5] Mahbubar Rahman,[5] Tahmeed Ahmed,[5] Monica Modesto,[8] Paola Mattarelli,[8] Clary B. Clish,[1] Hera Vlamakis,[1] Damian R. Plichta,[1,9] Olga Sakwinska,[4,*] and Ramnik J. Xavier[1,9,10,*]

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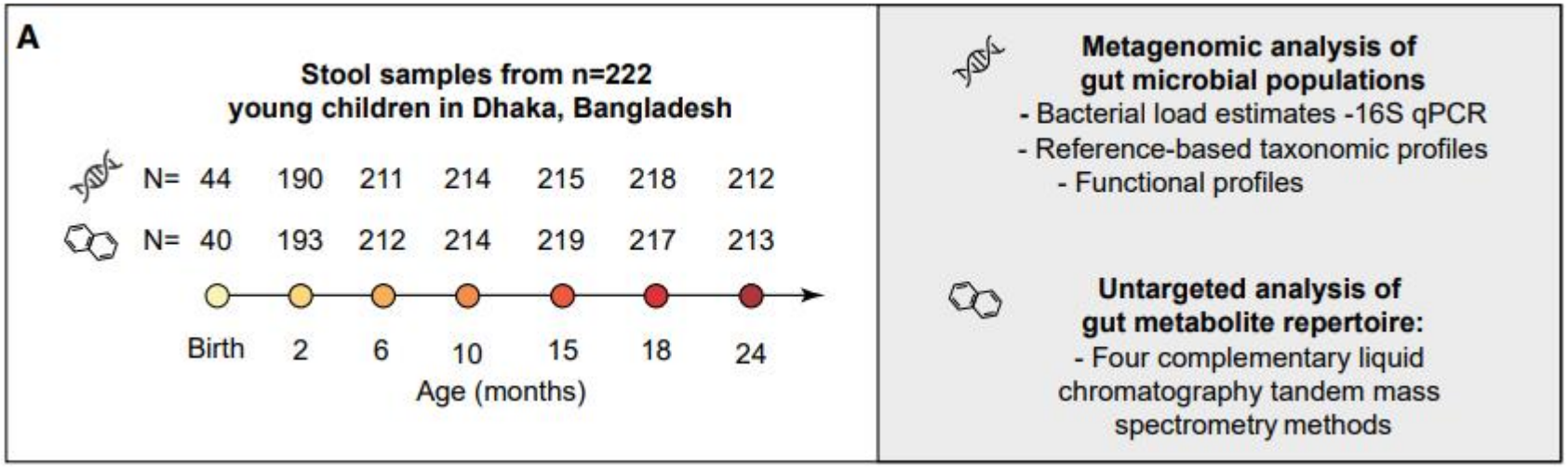
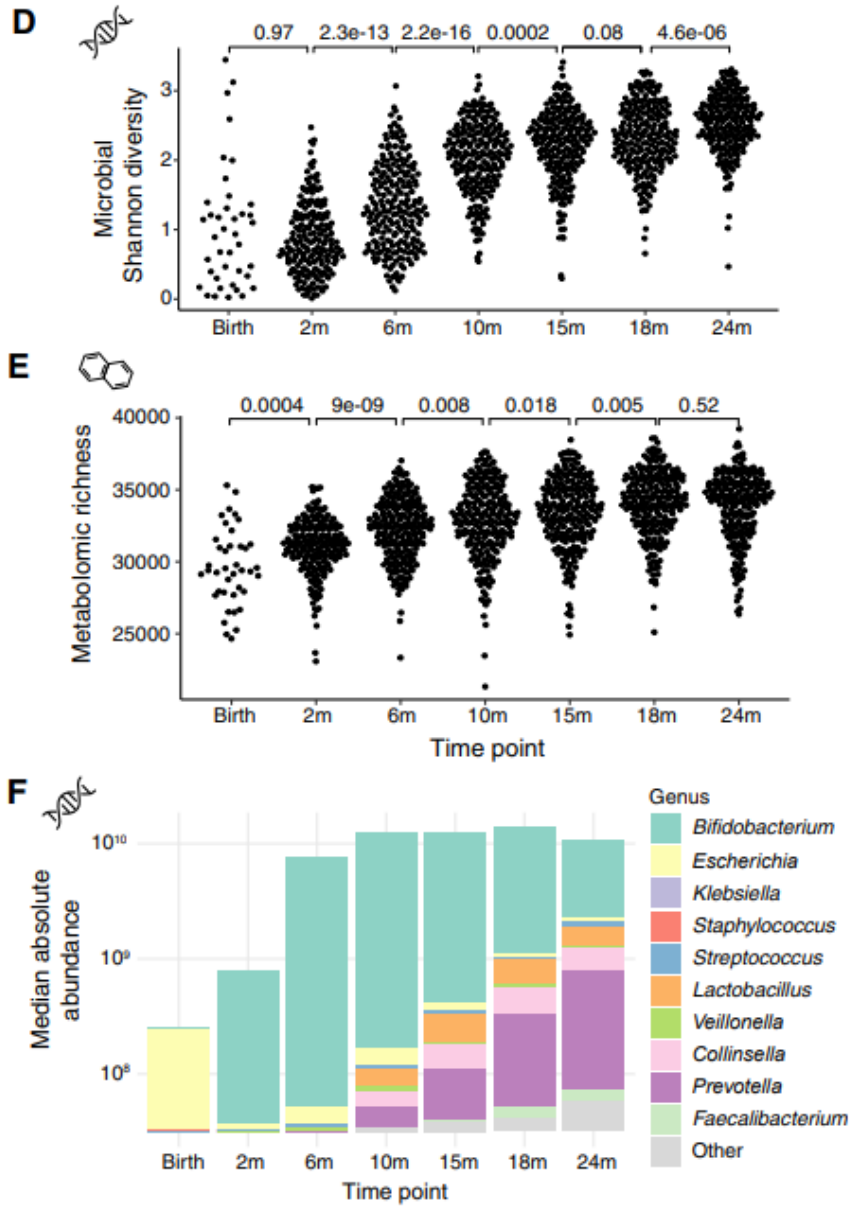
https://doi.org/10.1016/j.cell.2022.10.011

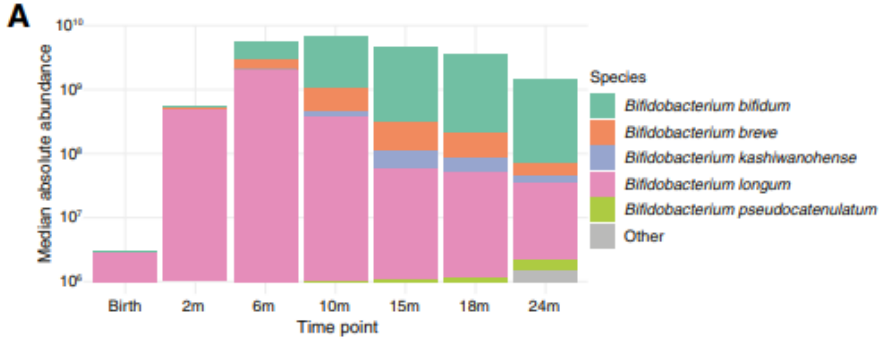
Cohort & experimental design

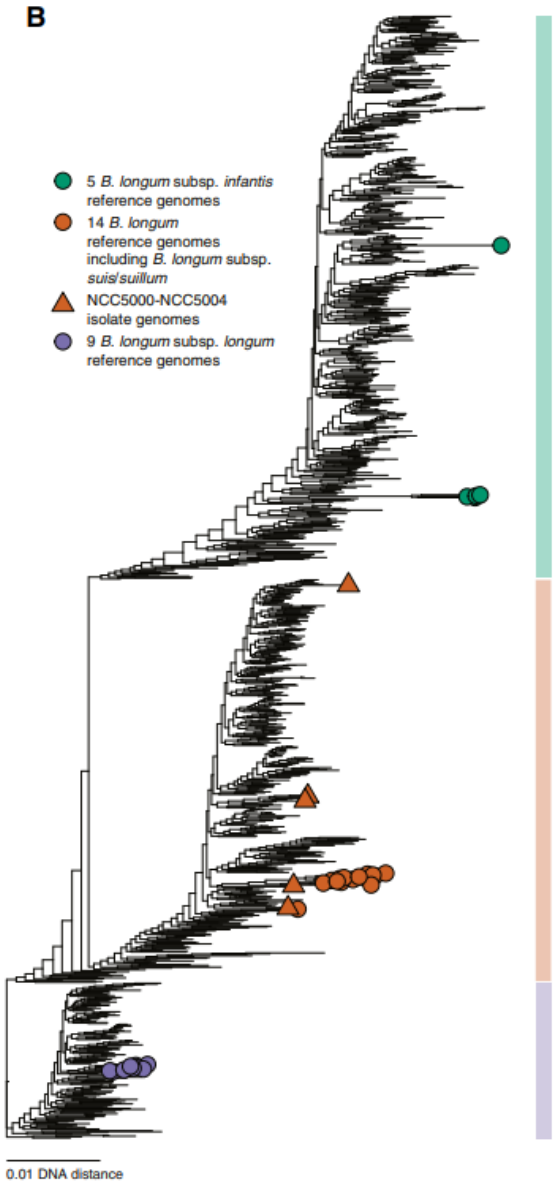


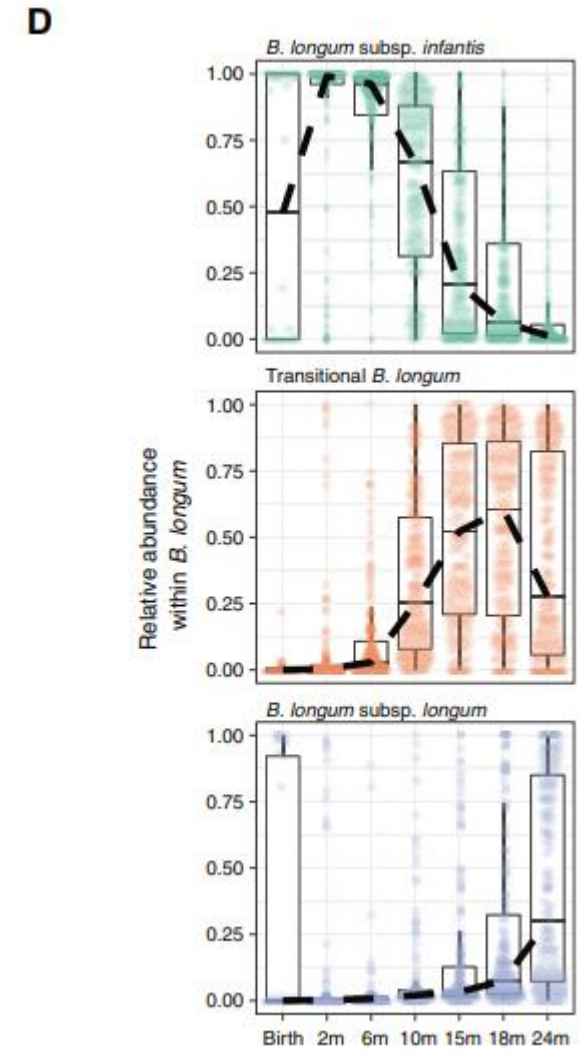
Profiling outcomes



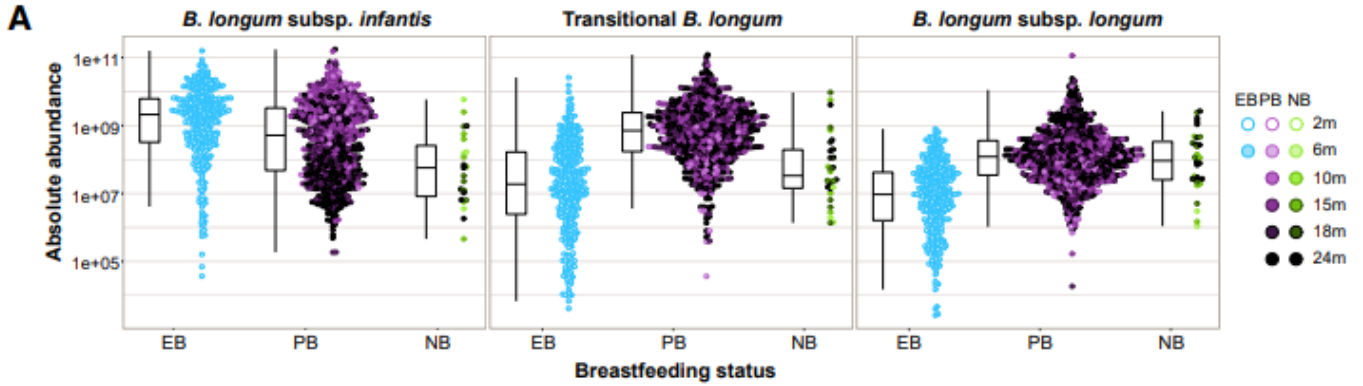
Three distinct *B. longum* clades identified

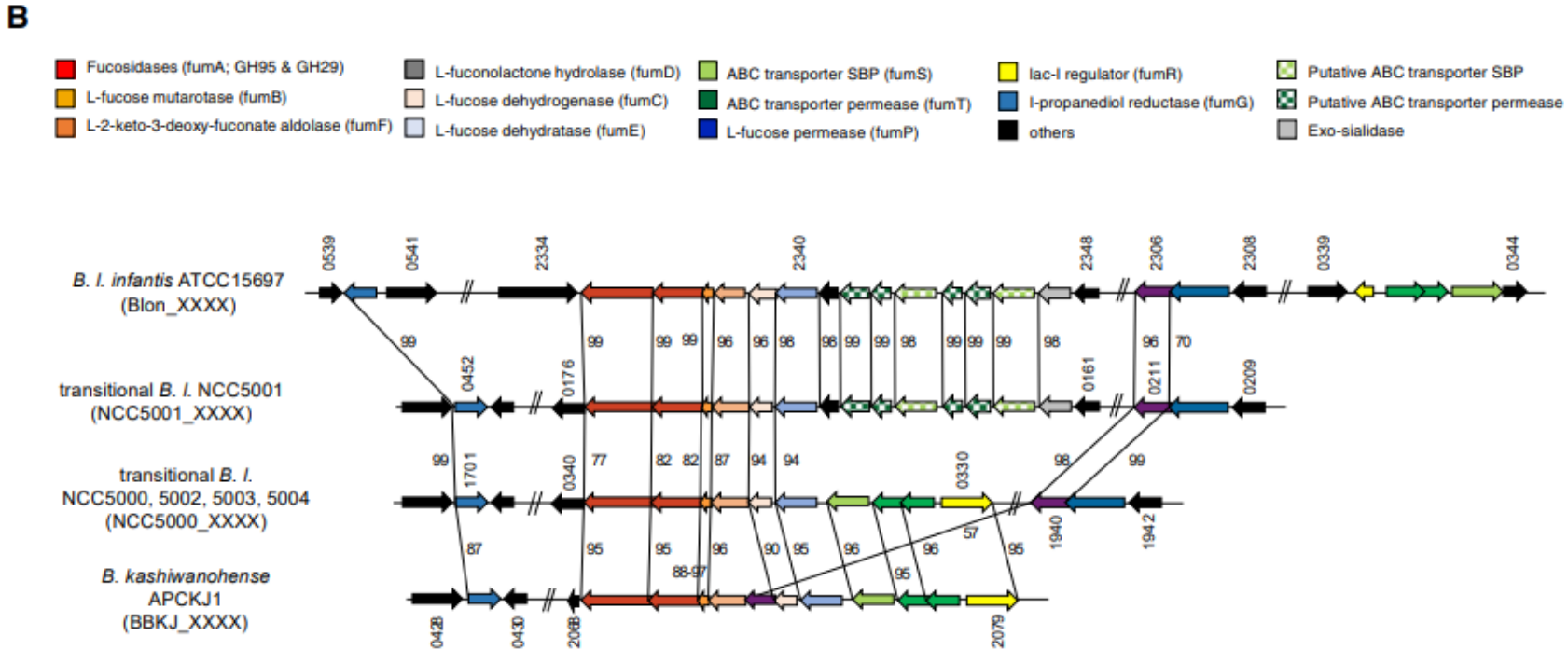




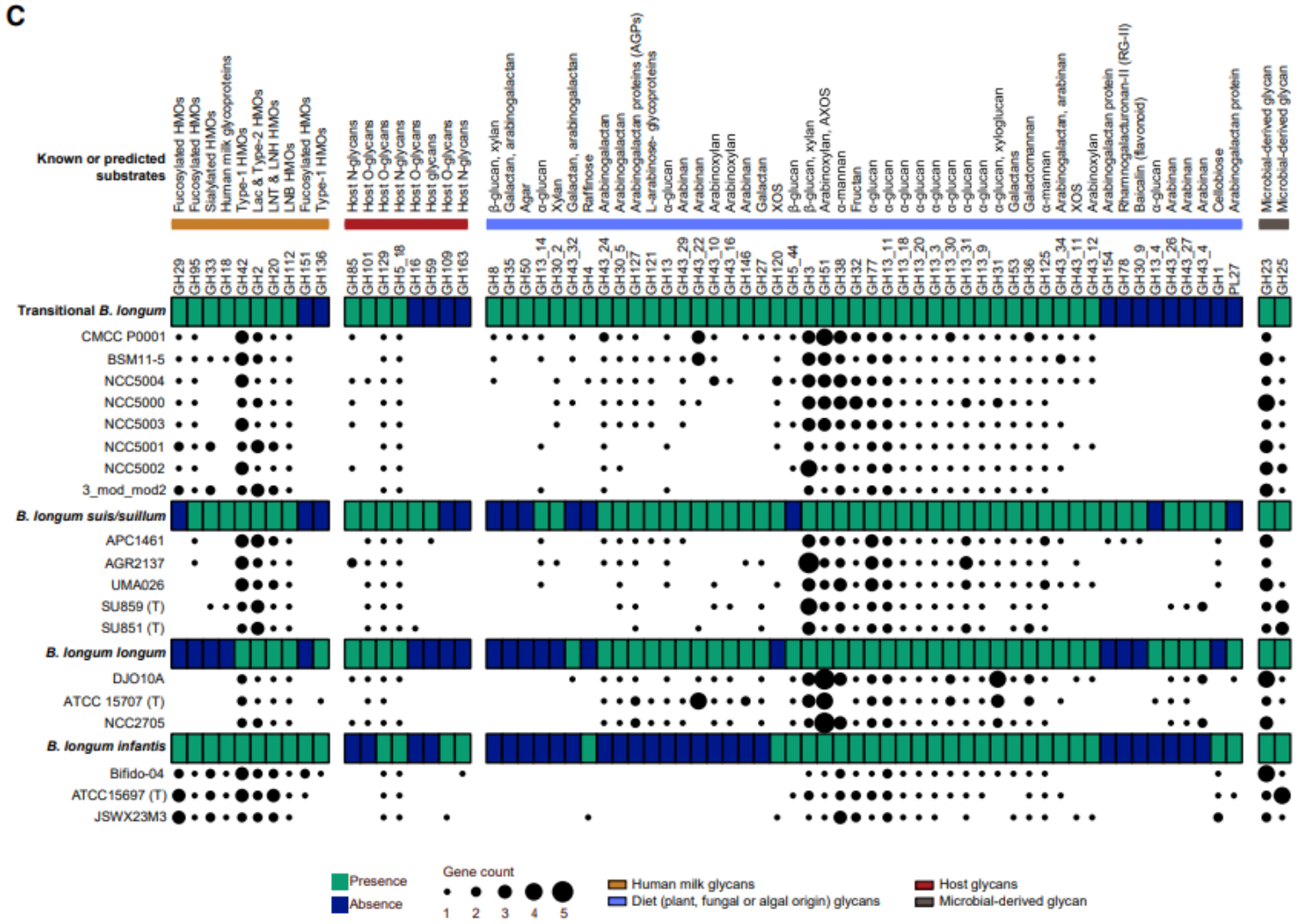


Transitional *B longum* dominant at weaning and harbors genes for metabolizing both HMO's and complex glycans





Transitional *B longum* metabolizes both HMO's and complex glycans



Conclusions

- Longitudinal metagenomics and metabolomics analysis of stool (from birth to 24 month)
- Complete profiling of microbial communities
- Identification of a new *B longum* clade
- This new clade can metabolize both HMO's and complex glycans
- Hypothesis: important for transition from breast feeding to solid food

Conclusions

- Genomics and proteomics technologies have tremendously evolved (and will continue to), stimmulated by:

- Knowledge of the genome and proteome, desire to discover new genomes
- Possibilities to ask questions that were not addressable in the past
- Unprecedented analysis of gut (and other organs) microbiome
- Move towards better understanding of molecular causes of health and disease
- Move towards more personalized (precision) medicine (diagnostics, clinics)
- Move towards more personalized nutrition
- Move towards improved agriculture (without necessarely GMO)

- Associated economical and ethical consequences to be considered as well