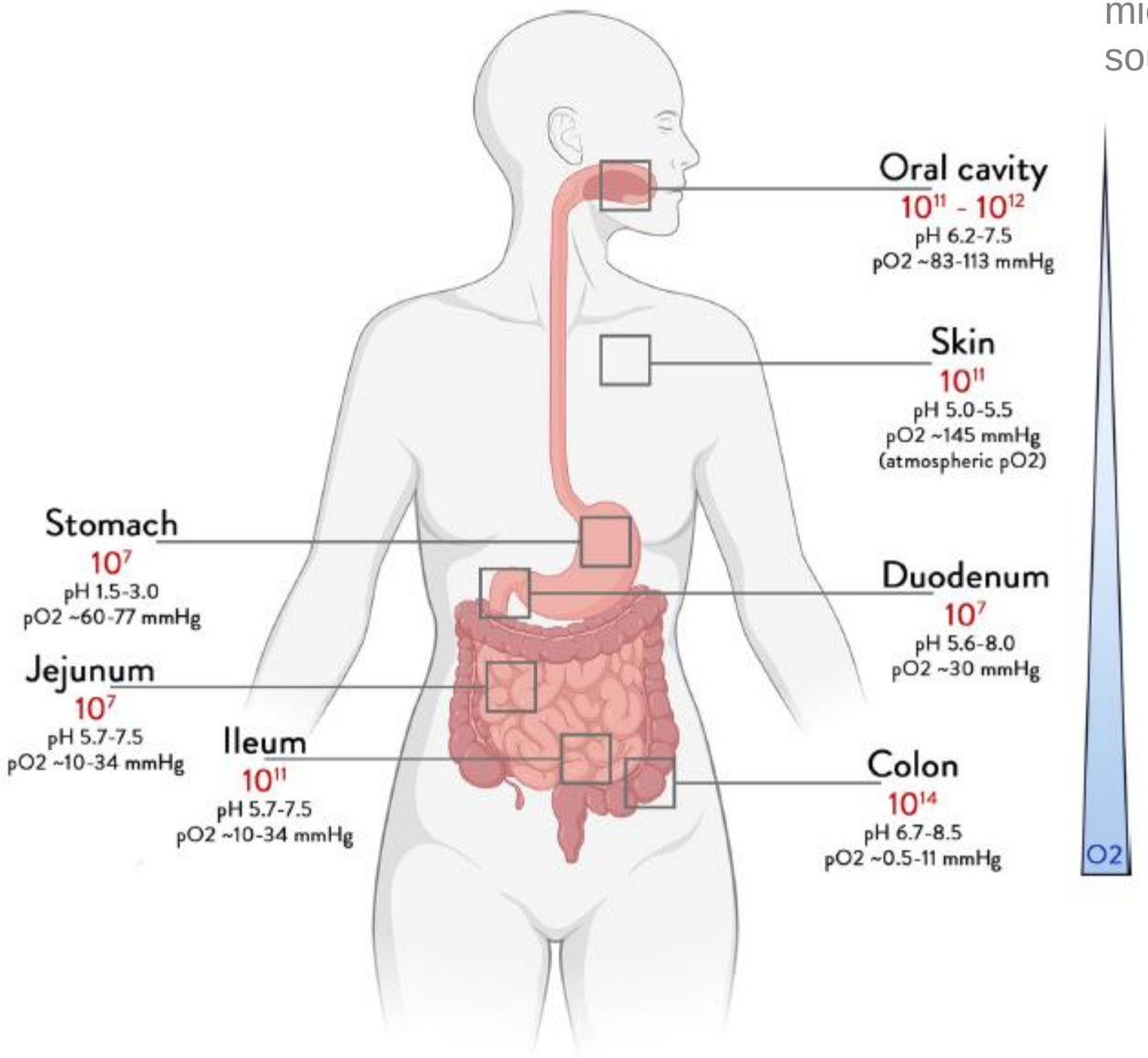
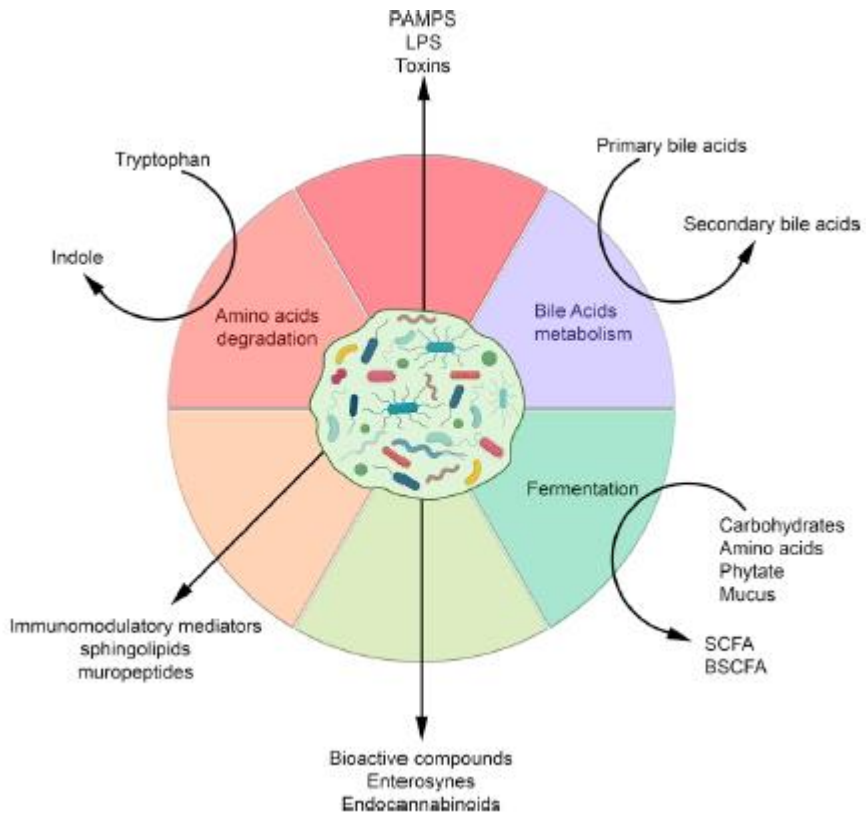
Total abundance of bacteria according to the different body sites



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 gut microbiome from birth to first years

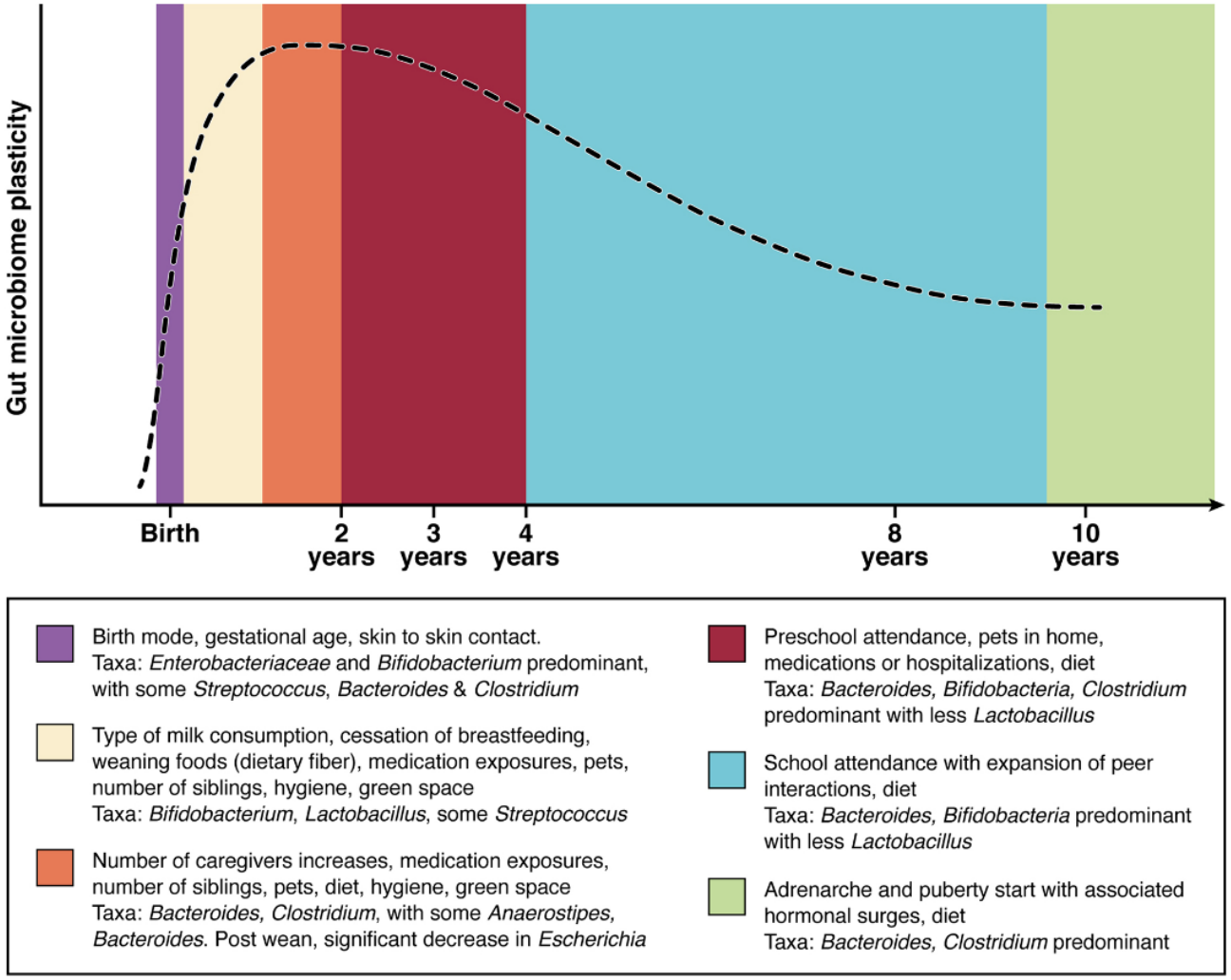
Clostridia, Enterobacteria, and Streptococci were observed in the infant intestines in the first 2 days after birth

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 1: 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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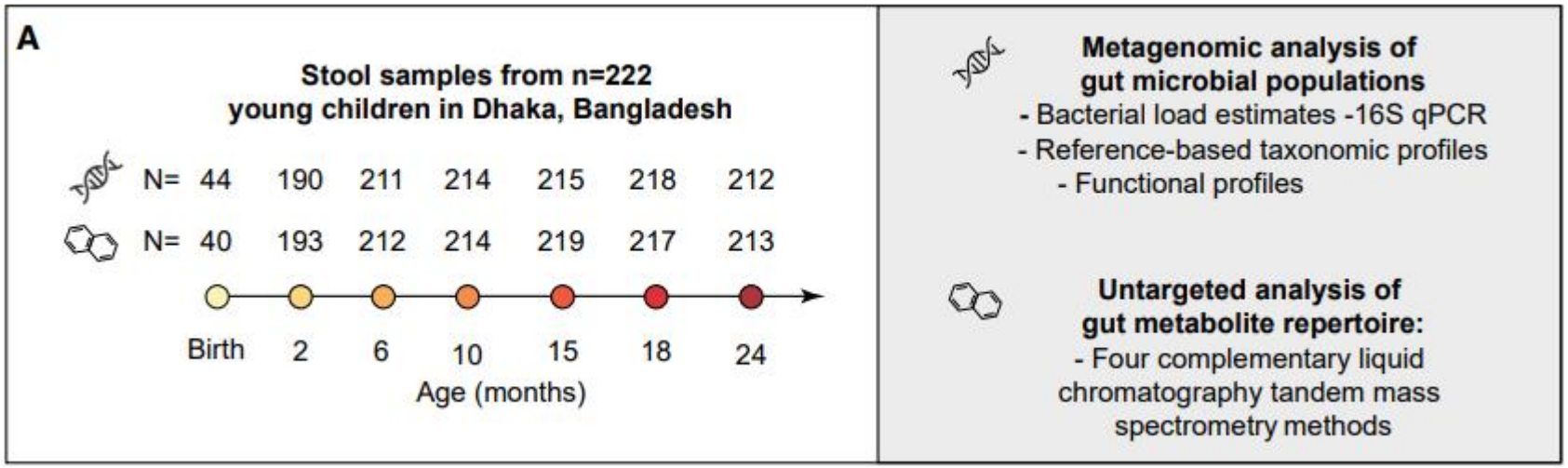
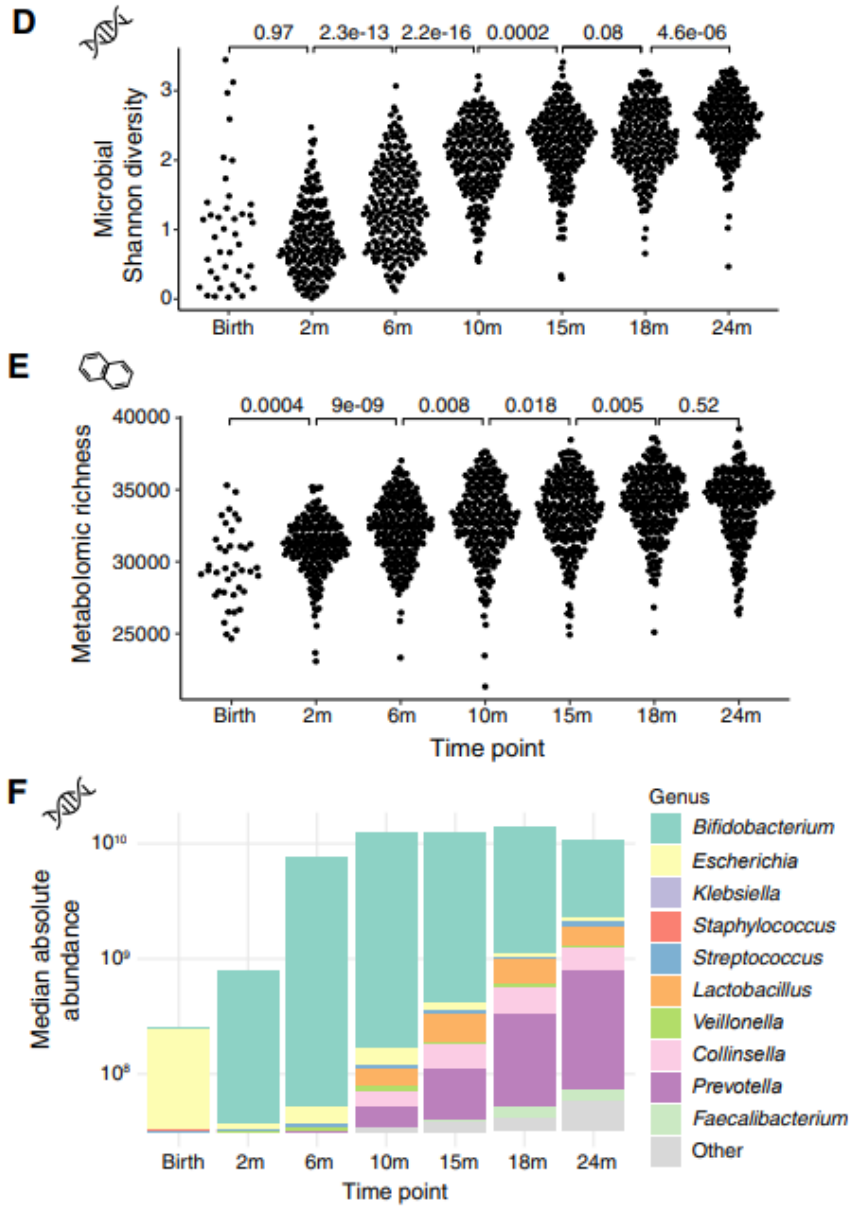
https://doi.org/10.1016/j.cell.2022.10.011

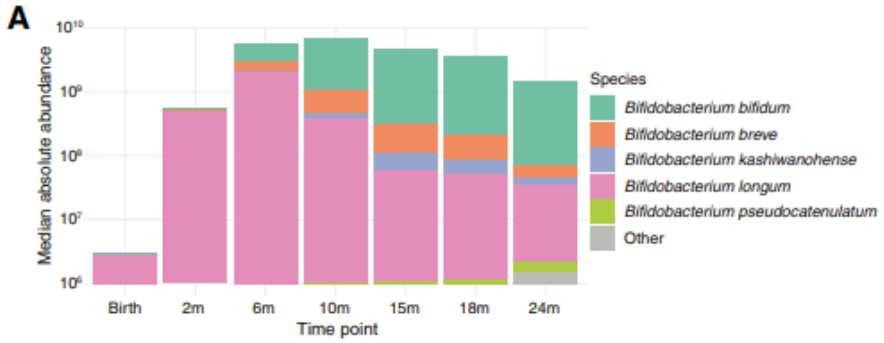
Cohort & experimental design

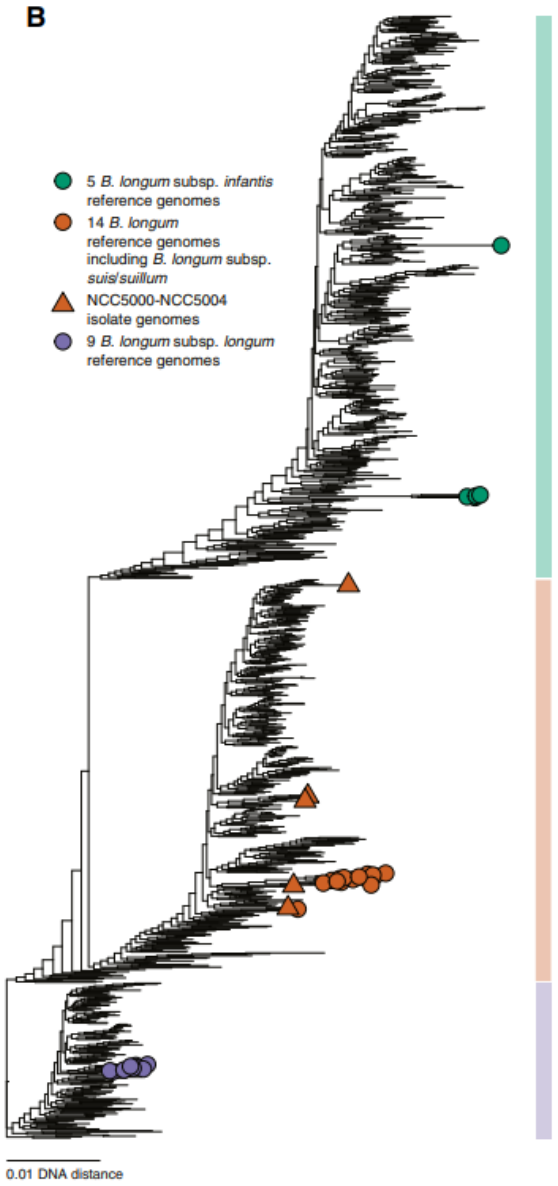


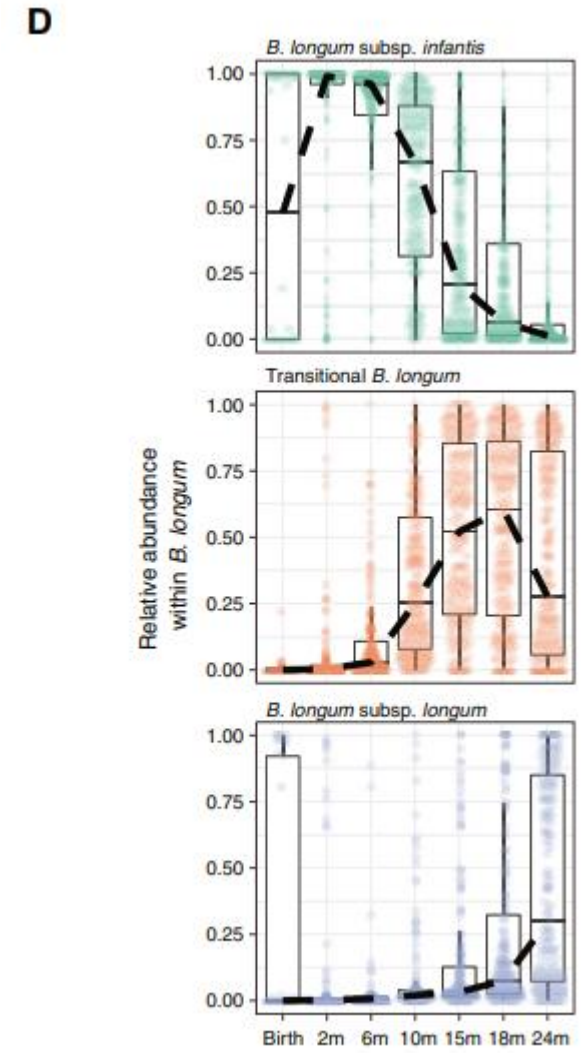
Profiling outcomes



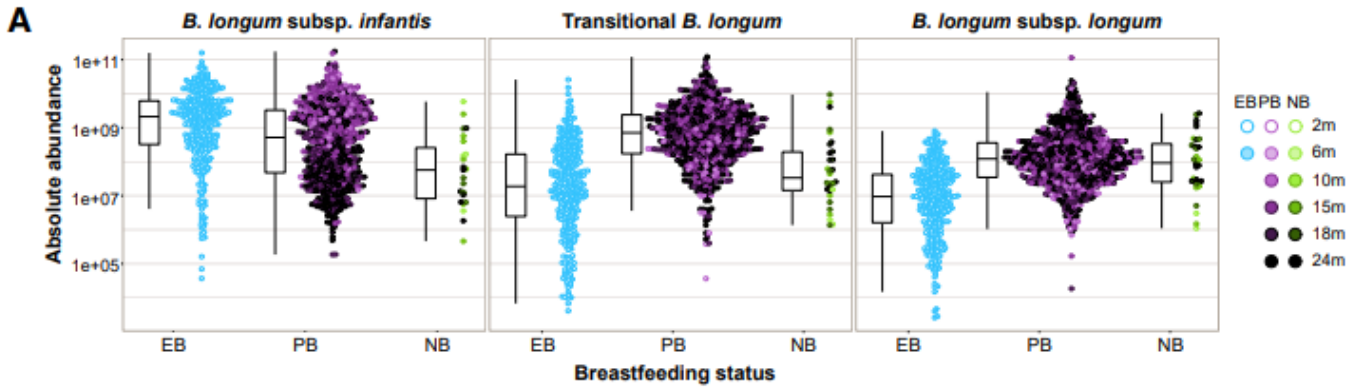
Three distinct *B. longum* clades identified

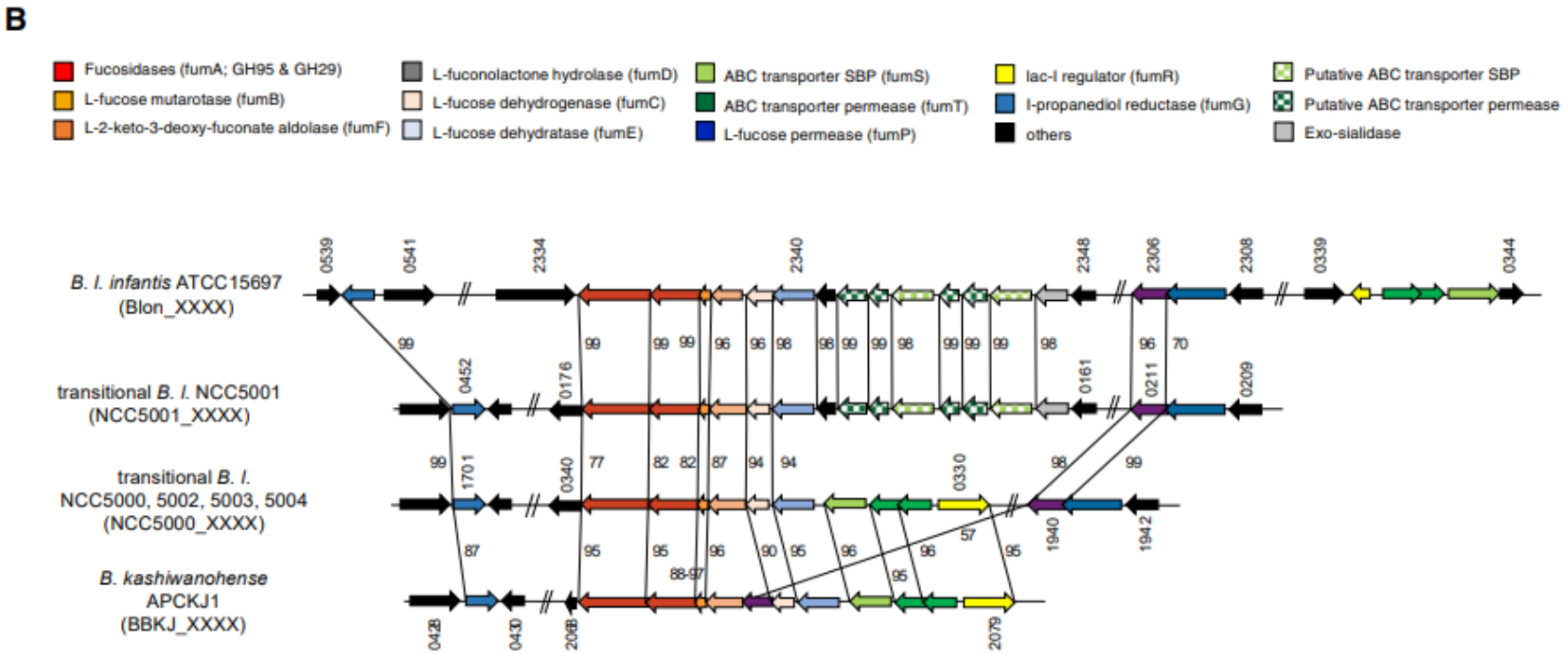




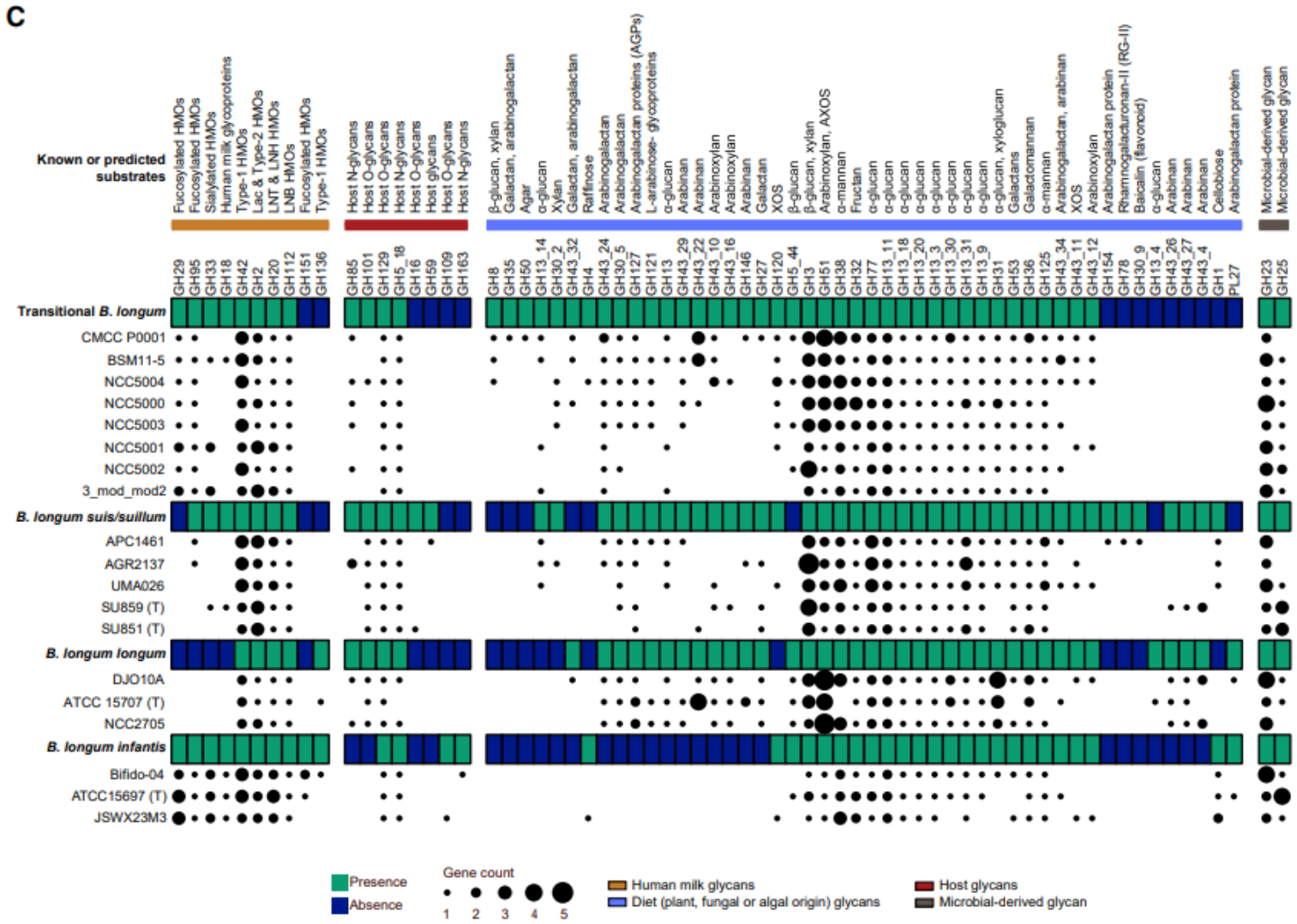


Transitional *B longum* dominant at weaning and harbors genes for metabolizing both HMO's and complex glycans





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
- Hyopthesis: important for transition from breast feeding to solid food

Microbiome and immunity

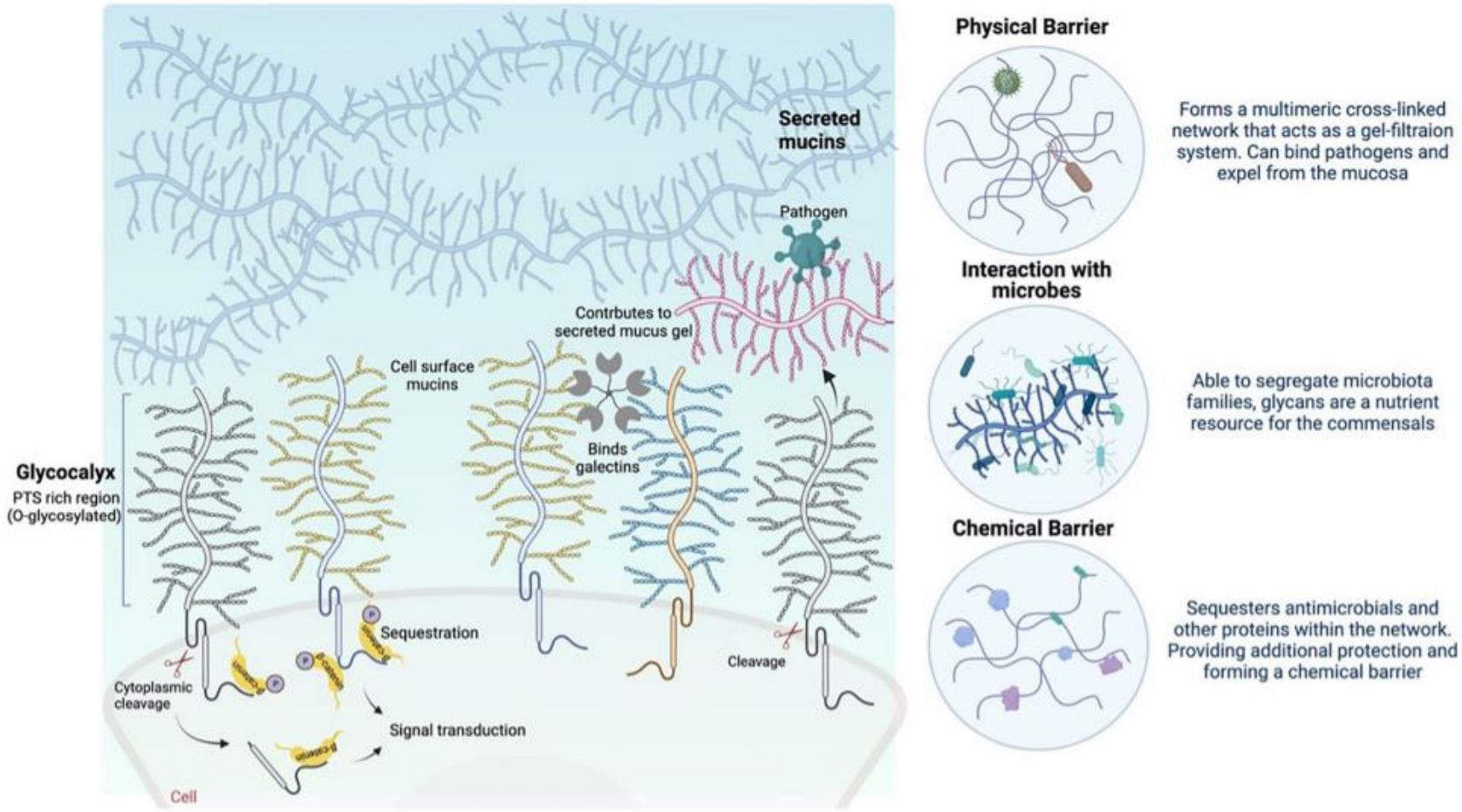
- From an ecological perspective, mammals and their commensal microorganisms co-evolved toward mutualism and hemostasis
- Early-life colonization of the mammalian host’s mucosal surfaces plays a pivotal role in maturation of the host’s immune system
- Immaturity of the immune system in newborns and infants is highlighted by an increased susceptibility to various infectious pathogens. rendering infectious diseases the leading cause for mortality in children.
- largest share of colonization occurs after birth, mainly originating from the maternal microbiota
- maternal antibodies delivered via breastmilk offer crucial passive protection against pathogens

Work on germ-free and laboratory mice strains

- absence of commensal microbes is associated with profound intestinal defects of lymphoid tissue architecture and immune functions.
- Intestinal microbial diversity during early-life colonization is critical to establish an immunoregulatory network that protects from induction of mucosal IgE, which is linked to allergy susceptibility

- Of note, the impact of the microbiome on immunity in laboratory mice can be vastly divergent from that in humans, which is in part explained by differences in microbiota between mice raised in laboratory versus wild environments. Wild mice have a microbiome more resilient to environmental challenges, and **more similar** to humans.

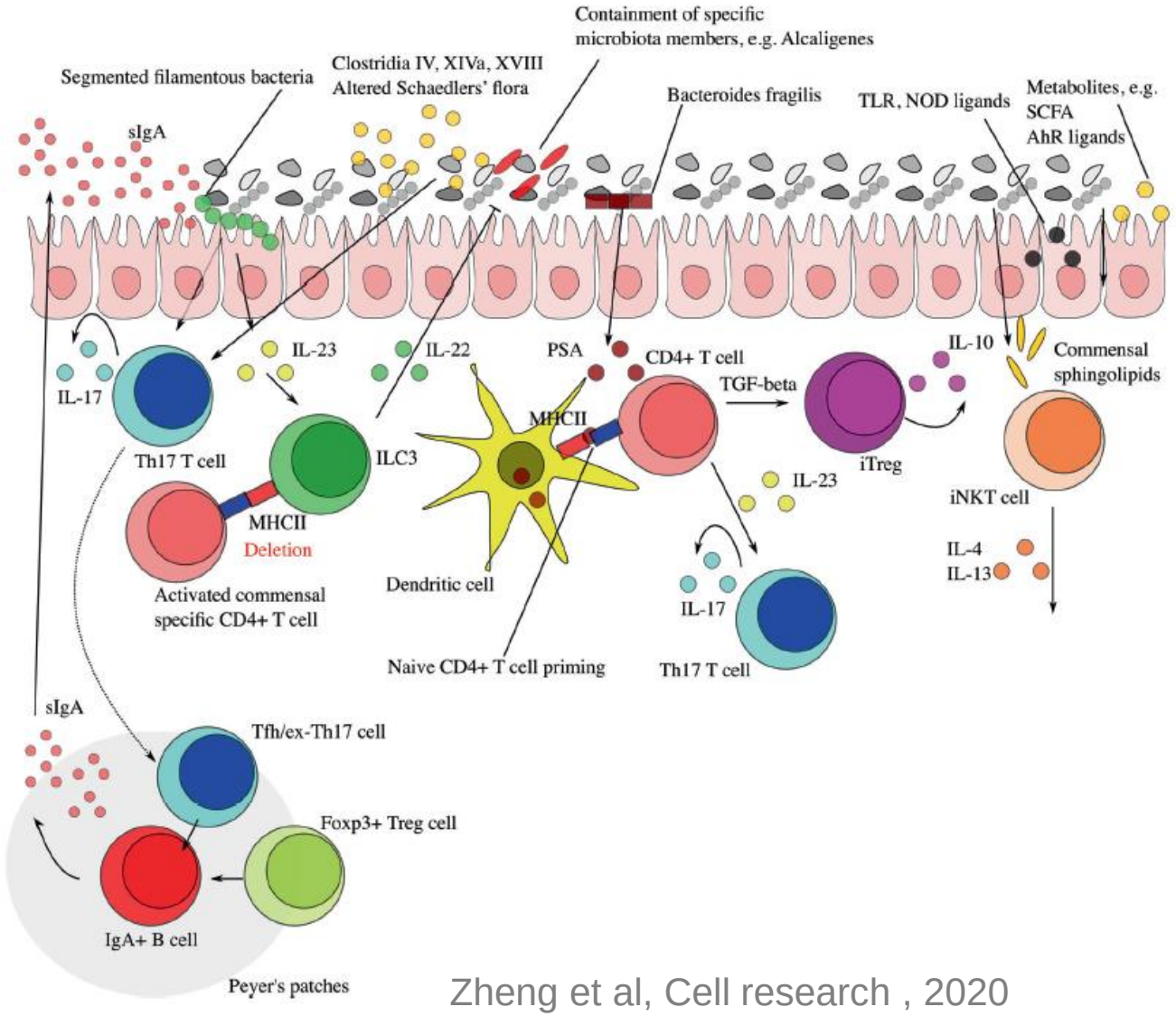
Role of cell surface & gel-forming mucins at the mucosal barrier



Sheng and Hasnain, Frontiers, 2022

Intestinal microbiota-immunity interplay in homeostasis

- Impacts on both innate and adaptive immune repsonses

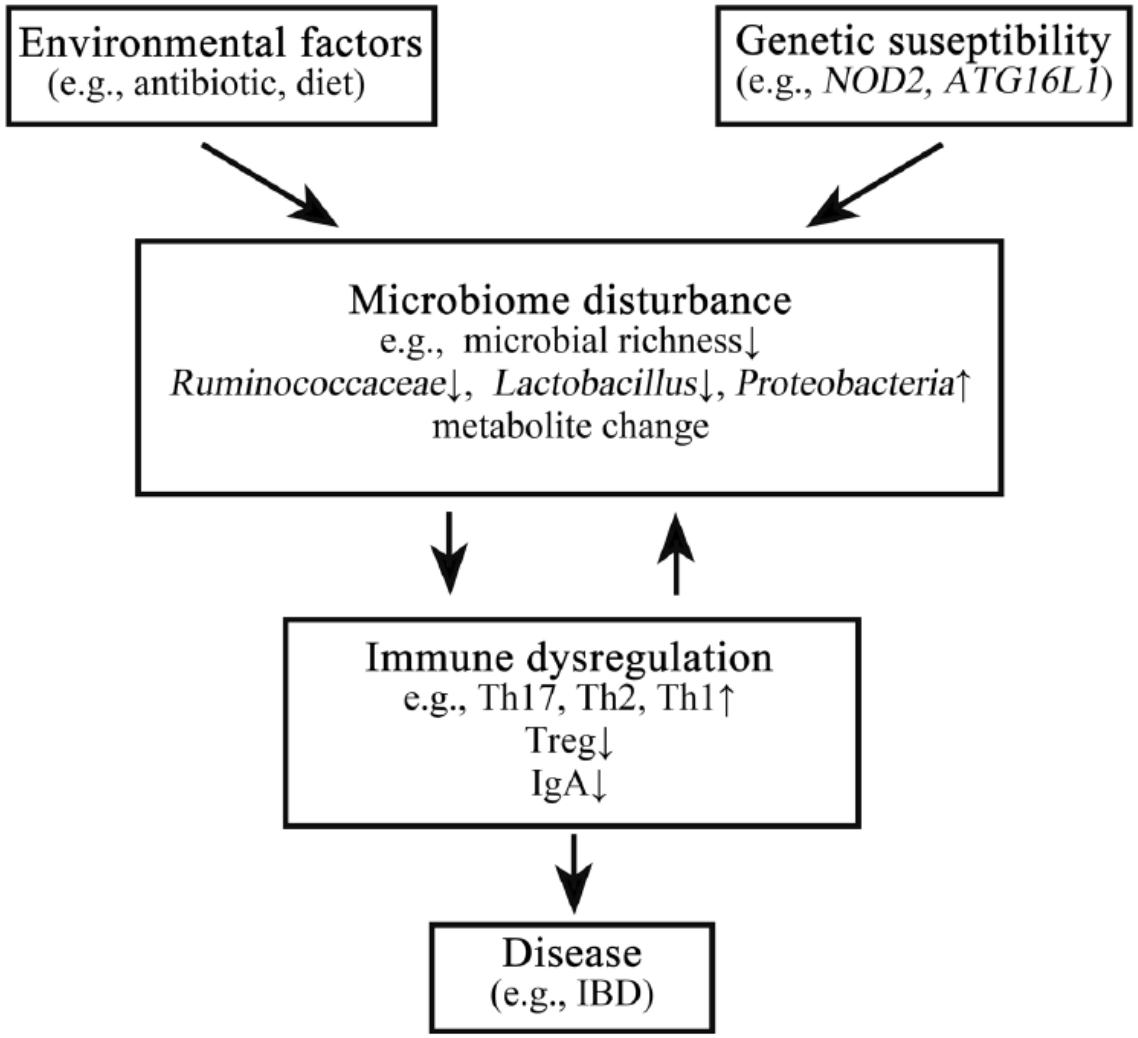


Zheng et al, Cell research , 2020

Influence of environmental microbiome perturbation on the immune system

- The gut microbiome is shaped by a wealth of environmental factors whose impacts dominate over host genetics
- diet, antibiotic use, westernized lifestyle, etc., are potential triggers of inflammatory and autoimmune diseases
- the best-studied environmental sources of microbiome variation are antibiotic treatment and diet

…and genetics plays also a role



Zheng et al, Cell research , 2020

Diet

General: gut microbiome mostly uses fibers and carbohydrates as source of energy.

Dietary fiber consists of non-starch polysaccharides and other plant components such as cellulose, resistant starch, resistant dextrins, inulin, lignins, chitins (in fungi), pectins, beta-glucans, and oligosaccharides

- diet high in saturated fats increases the levels of taurocholic acid, a secondary bile acid, and in turn fosters the expansion of *Bilophila wadsworthia*
- High-fat diet can also aggravate disease severity in chemically induced murine colitis by disturbing the homeostasis of intestinal DCs
- the timing of dietary intake has been recently shown to affect microbiome composition and in turn immunity

Antibiotics

- antibiotic use during childhood is associated with the development of a range of immune-mediated diseases, including allergies and IBD
- antibiotic affects the composition and function of the gut microbiota, and may introduce long-lasting adverse effects on the host
- Inhibition of mucosal mast cells activation
- hyperactivation of intestinal macrophages and expansion of proinflammatory T helper cells and increases susceptibility to infection

Some examples of dysregulation of microbiome-immunity interaction in diseases

- Inflammatory bowel disease Crohn's disease (CD) and Ulcerative colitis (UC).
- reduced bacterial diversity and marked shifts in abundance of certain bacterial taxa, including decreased abundance of *Bacteroides, Firmicutes, Clostridia, Lactobacillus, Ruminococcaceae* and increased abundance of *Gammaproteobacteria* and *Enterobacteriaceae*, coupled with altered microbiome associated metabolite profiles
- disruptions of gut barrier integrity, including the mucus layer and epithelial cell junctions leads to translocation of bacterial symbionts into the mucosal layer, fueling aberrant host immune responses and tissue injury
- Genetics. GWAS: > 200 genes associated with IBD. NOD2 is an intracellular PRR capable of recognizing bacterial peptidoglycan-conserved motifs. NOD2 acts as a critical regulator of the intestinal commensal microbiota. Mutations in NOD2 dysregulate microbiome-immunity and contributed to CD

Some examples of dysregulation of microbiome-immunity interaction in diseases

- Rheumatoid arthritis
- Cardiometabolic diseases (obesity, T2D, atherosclerosis and non-alcoholic fatty liver disease (NAFLD)) where chronic low-grade inflammation is considered a hallmark of these metabolic disorders. gut microbiome-derived metabolites can reach systemic circulation through the gut barrier and fuel metabolic inflammation
- Cancer

Some examples of cross talks between microbiota and immunity and extra-intestinal organs

Skin:

- skin microbiota induces protective and regulatory immunity that contributes to host-microbe mutualism
- One of the most highly abundant skin commensals, *Staphylococcus epidermidis*, involved in wound healing
- Skin dysbiosis has been associated with different inflammatory skin disorders, including atopic dermatitis and psoriasis

Lung:

- The human microbiome in the lower respiratory tract forms within the first 2 postnatal months, alongside lung immune maturation. Alterations of the lung microbiota has been implicated in exacerbation of chronic pulmonary diseases, including chronic obstructive pulmonary disease, asthma and cystic fibrosis.

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

Case study 2: shaping the gut microbiome composition

Article

Microbial transformation of dietary xenobiotics shapes gut microbiome composition

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https://doi.org/10.1016/j.cell.2024.08.038

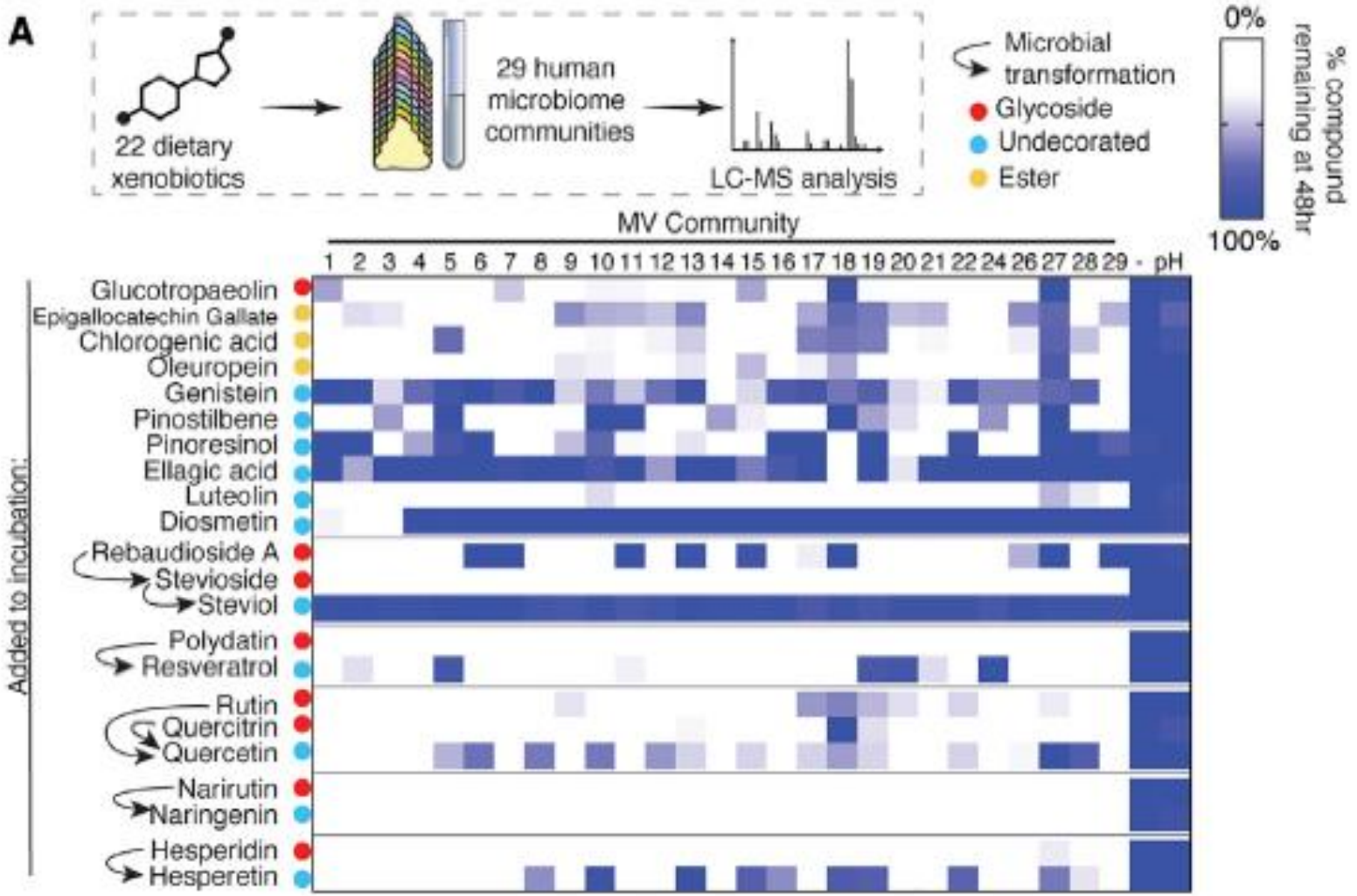
- Cell 187, 6327-6345 Oct 31st 2024

Rationale

- Human subject experience significant interindividual variability in response to the same food
- Diet is a major determinant of gut microbiome composition
- Diet is composed of macro- (fats, proteins, carbohydrates) and micro-nutrients (for example > 26’000 xenobiotics such as polyphenols, lignenes, stilbenes and tannins)
- Question: how better understand the interactions between gut microbiome and dietary xenobiotics?

Growth impact and metabolism of dietary xenobiotics

- Microbiome of 29 healthy donors
- 22 representative xenobiotics
- Each compound incubated with human microbiome samples in anaerobic conditions in fermenters
- Metabolites measured by LC-MS


A
22 dietary xenobiotics
29 human microbiome communities
LC-MS analysis
Microbial transformation
Glycoside
Undecorated
Ester
0%
100%
% compound remaining at 48hr
MV Community
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 24 26 27 28 29 - pH
Added to incubation:
Glucotropaeolin
Epigallocatechin Gallate
Chlorogenic acid
Oleuropein
Genistein
Pinostilbene
Pinoresinol
Ellagic acid
Luteolin
Diosmetin
Rebaudioside A
Stevioside
Steviol
Polydatin
Resveratrol
Rutin
Quercitrin
Quercetin
Narirutin
Naringenin
Hesperidin
Hesperetin

Impact of a larger set of 161 compounds on growth of 26 individual bacterial species

- Definition of compounds toxicity
- 50% compounds have no strong

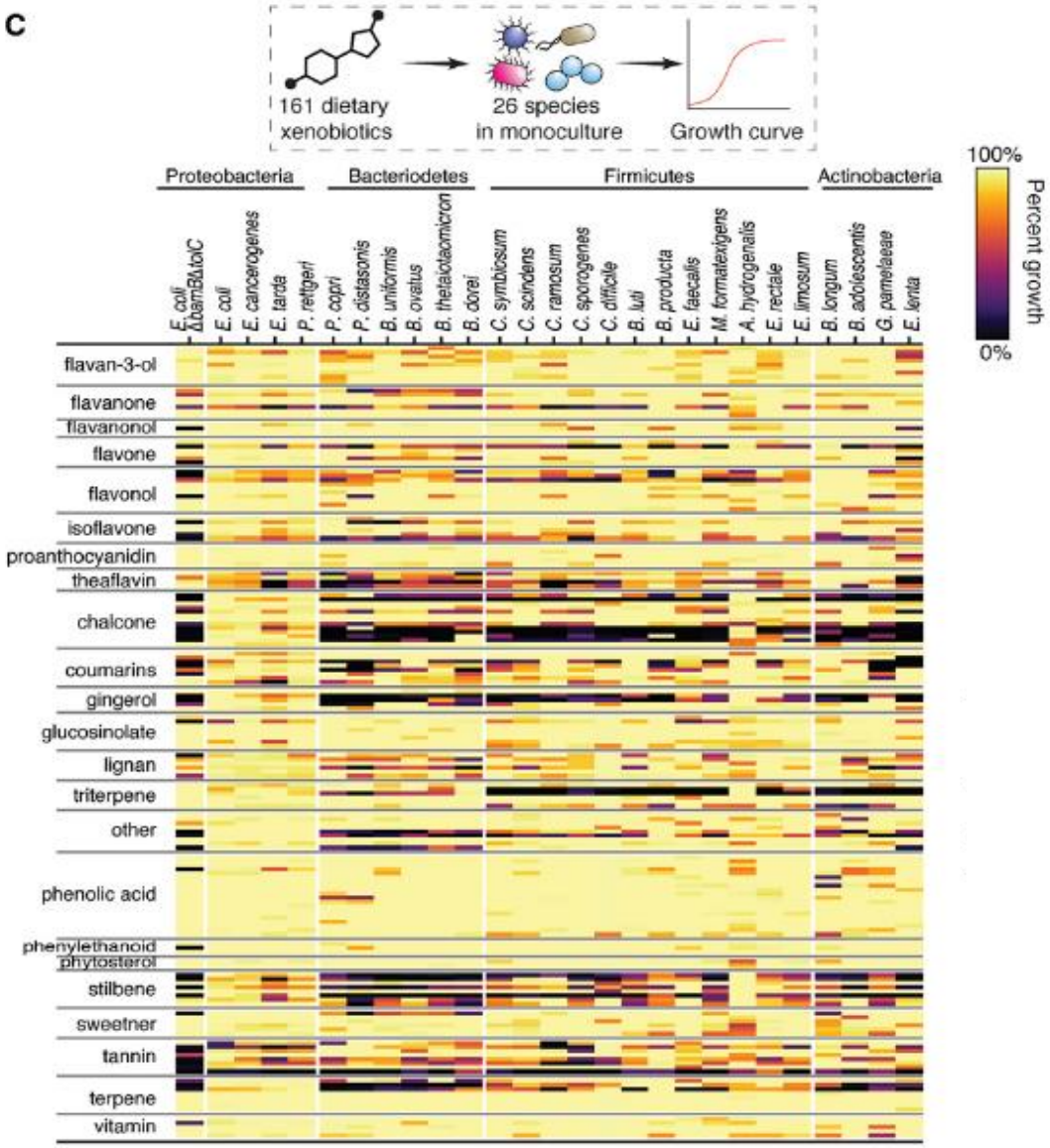
Inhibitory effect

- 9% inhibit growth of more than half

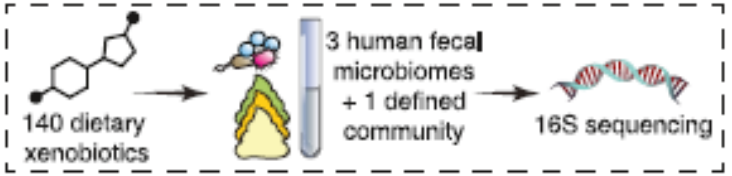
of the species

- 32% inhibit at least 50% of growth of

at least 2 species



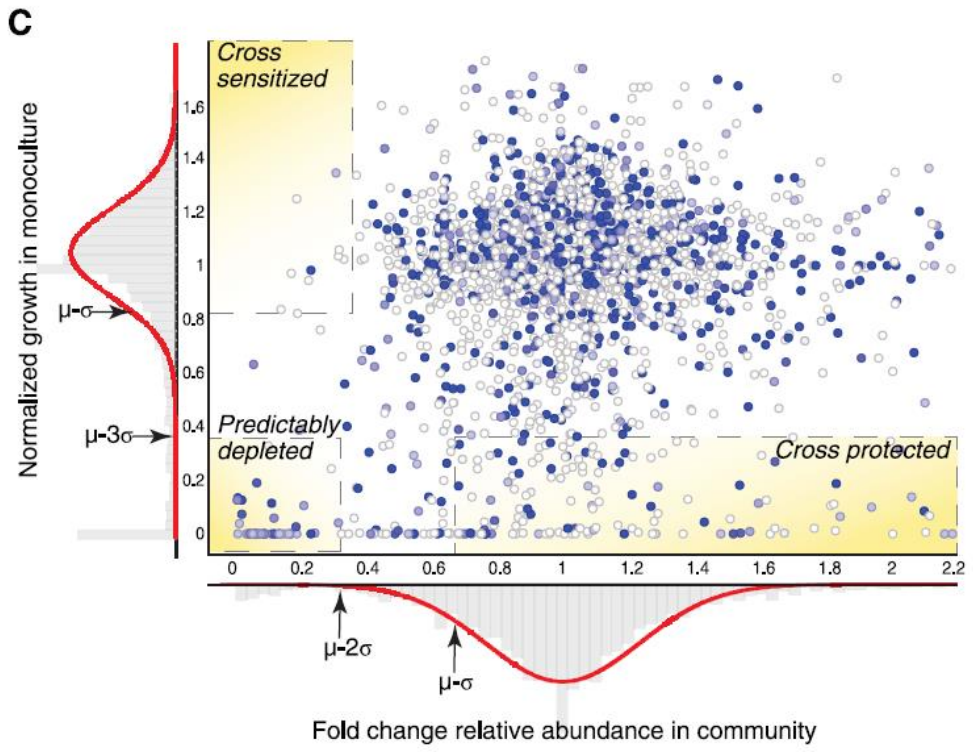
Xenobiotics remodel composition of gut microbial communities



- 140 xenobiotics
- 3 human communities
- One 38-member defined community
- 16S rRNA profiling to identify changes in communities
- Some compounds significantly impact the global microbial diversity

Individual species susceptibility vs relative abundance in the community

- Cross-sensitization: species reduced in the community but not in monoculture
- Cross-protecion: species reduced in monoculture but not in the community



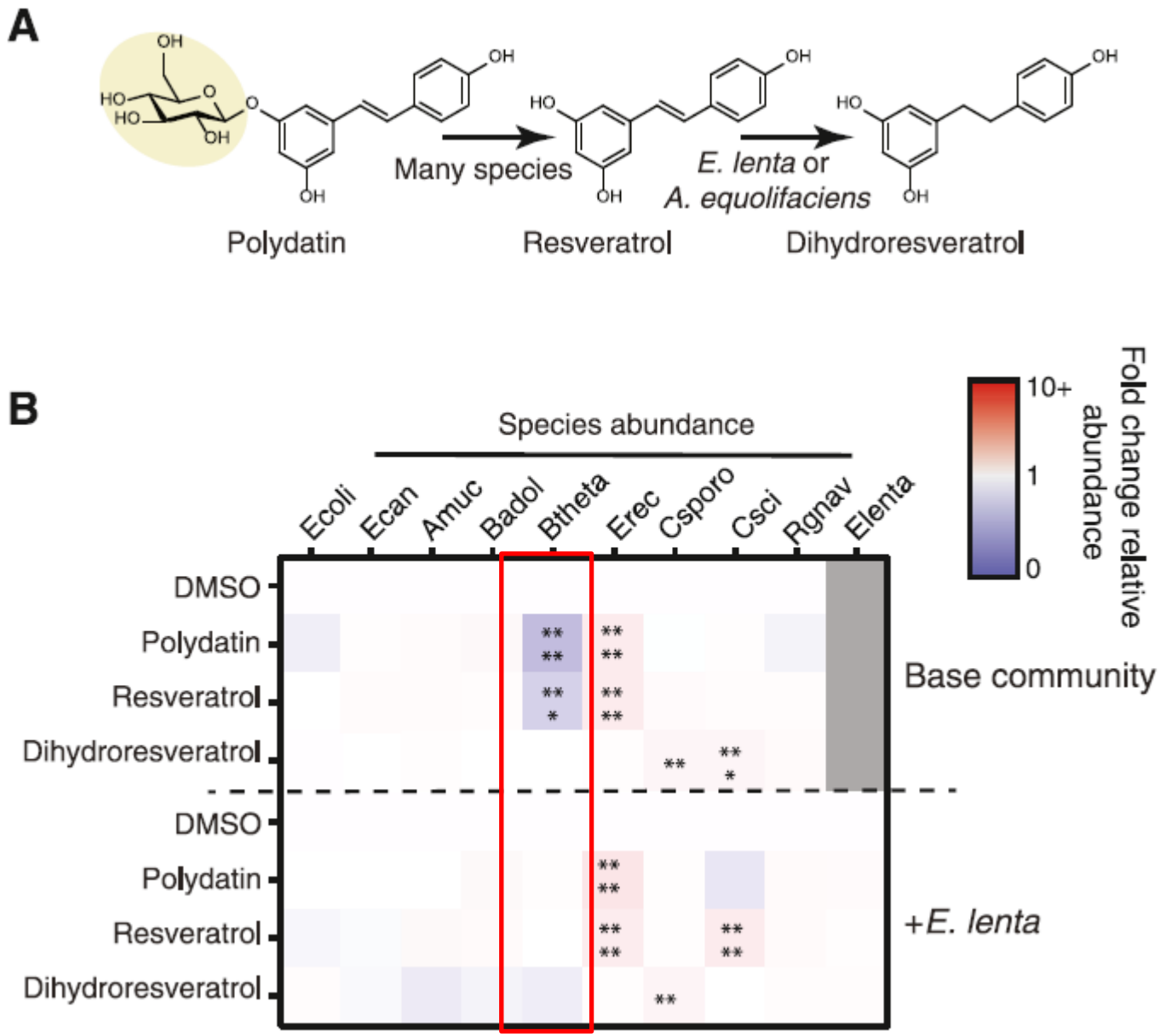
Hypothesis

Dietary xenobiotics interact with the gut microbiome to explain emergent properties (cross-sensitization/cross-protection) and variability in the effects of a compound on different communities.

Hypothesis:

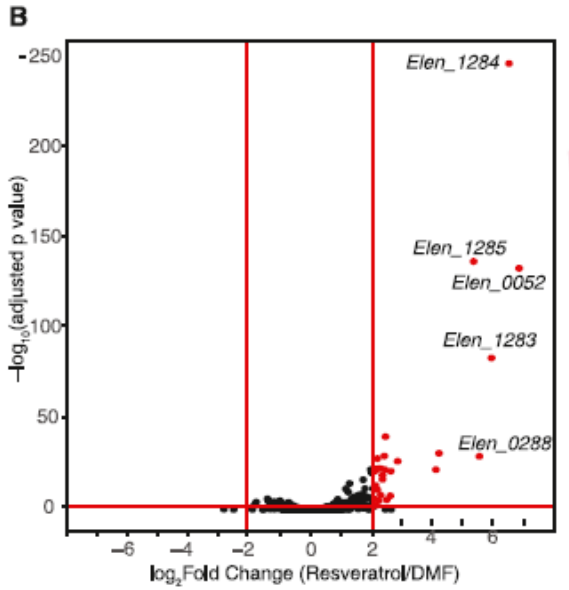
- Microbial metabolism of non-toxic dietary xenobiotics could produce toxic metabolites that deplete susceptible species, thus resulting in cross-sensitization.
- Microbial metabolism could detoxify otherwise toxic dietary xenobiotics, resulting in cross-protection of susceptible species.

Test with resveratrol

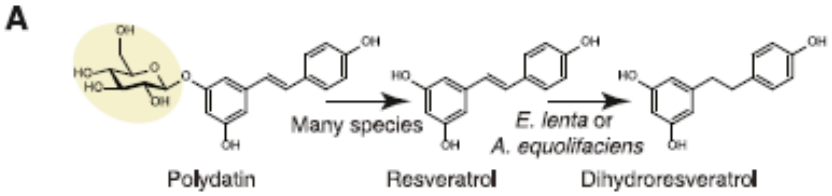


Identifying resveratrol metabolizing enzymes

- Gene expression of *E. lenta* +/- resveratrol
- Role of Elen_288 gene



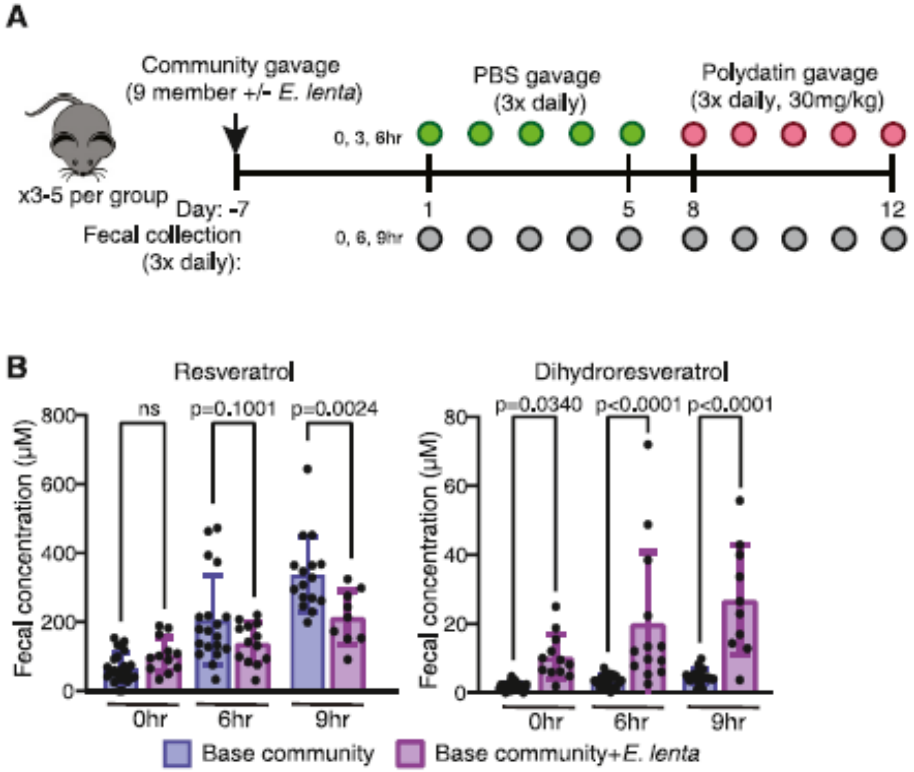
In vivo community remodelling

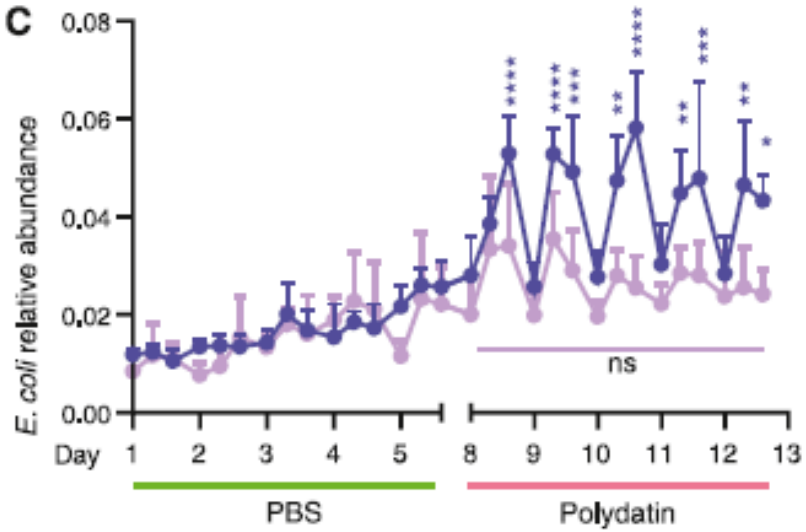


Germ-free mice colonized with members community +/- *E. lenta*

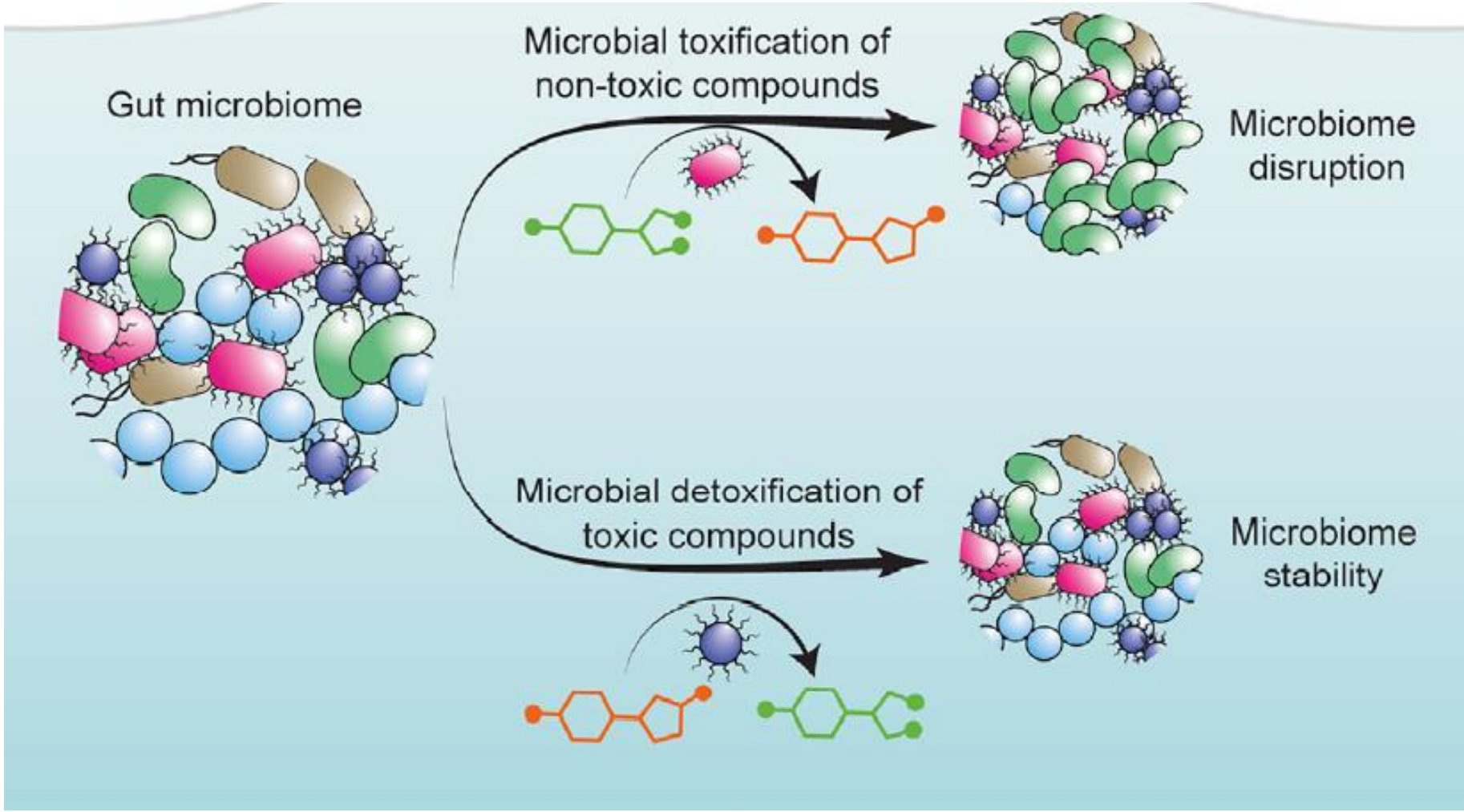
=> Presence of *E. lenta* alters exposure to polydatin

& protects the community incl. *E. Coli*





Summary


Gut microbiome
Microbial toxification of non-toxic compounds
Microbiome disruption
Microbial detoxification of toxic compounds
Microbiome stability

Toxicology

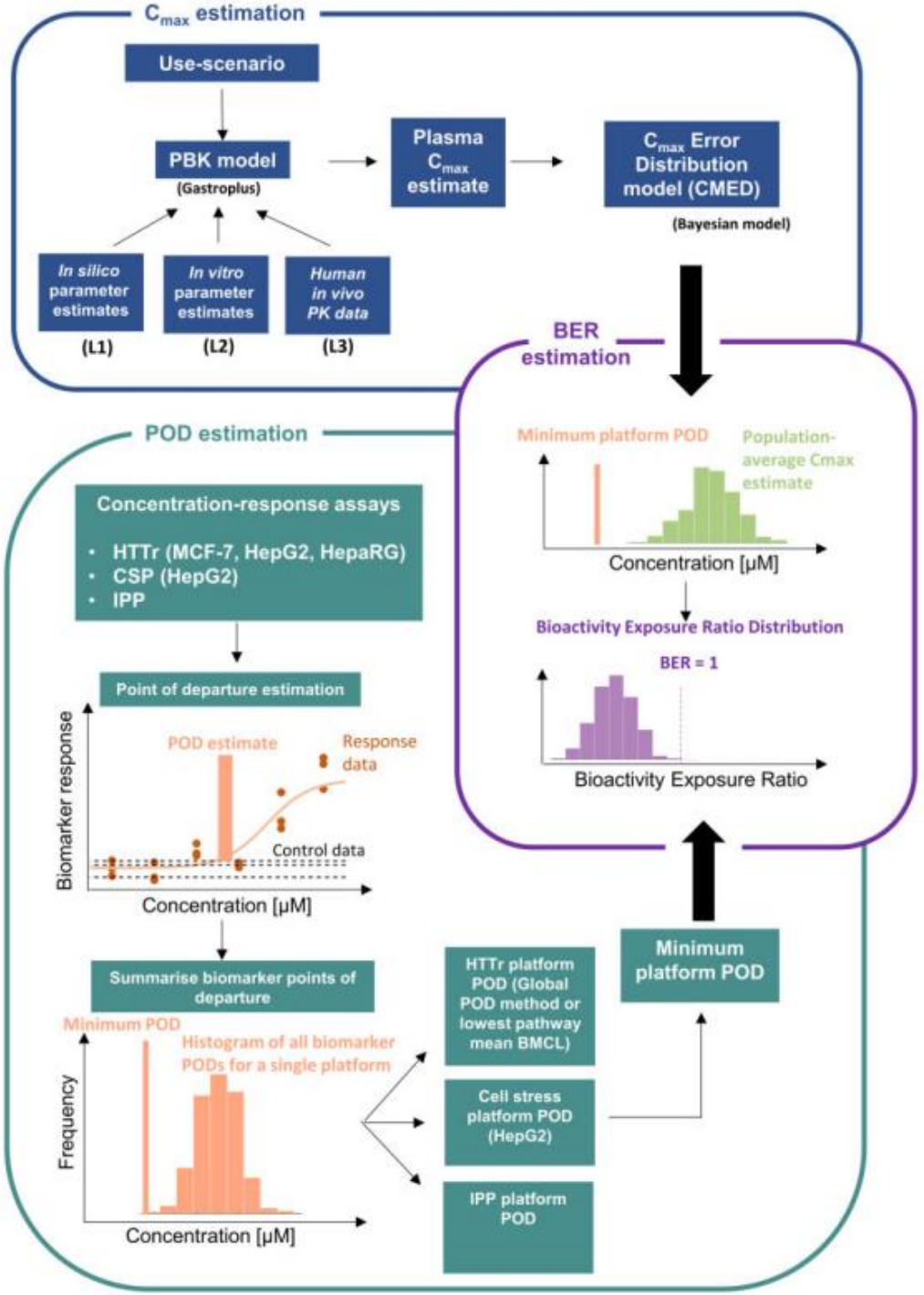
- Food toxicology deals with the substances found in food that, when consumed, may cause harm to the consumers
- Key to assess toxicology to ensure safety of food and beverages
- Examples of some areas/types of potential toxic compounds:
  - diet on body weight and health outcomes including results from animal models of carcinogenesis
  - methods for microbial oil extraction
  - food processing and its impact on food safety and health
  - novel compounds to avoid mycotoxin contamination of agricultural products
  - safety of cannabidiol in food supplements based on Cannabis sativa extracts

Models used for toxicology evaluation

- one rodent (e.g., rat, mouse)
- non- rodent (e.g., dog, nonhuman primate)
- Biologics may require only one species.
- Other species (e.g., rabbits, ferrets, hamsters, mini- pigs)

- Non –animal models more and more developed and preferred

- point of departure (POD) based on the cell stress panel (CSP)
- high throughput transcriptomics (HTTr)
- bioactivity exposure ratio (BER)



Middleton et al, Toxicological Sciences, 2022

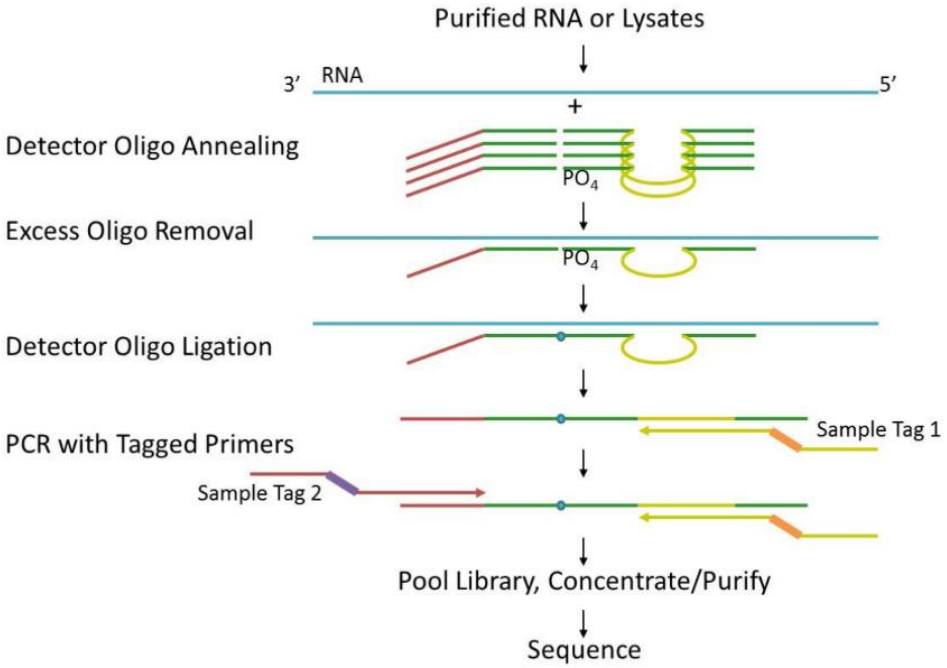
In vitro models

- Hepatic cell lines (as liver is the main organ involved insesing and metabolizing drugs/toxic compounds)
- Application of dose response (increasing concentrations of compounds)
- Transcriptome profiling
- Pathway analysis in relation to toxicological responses/genes

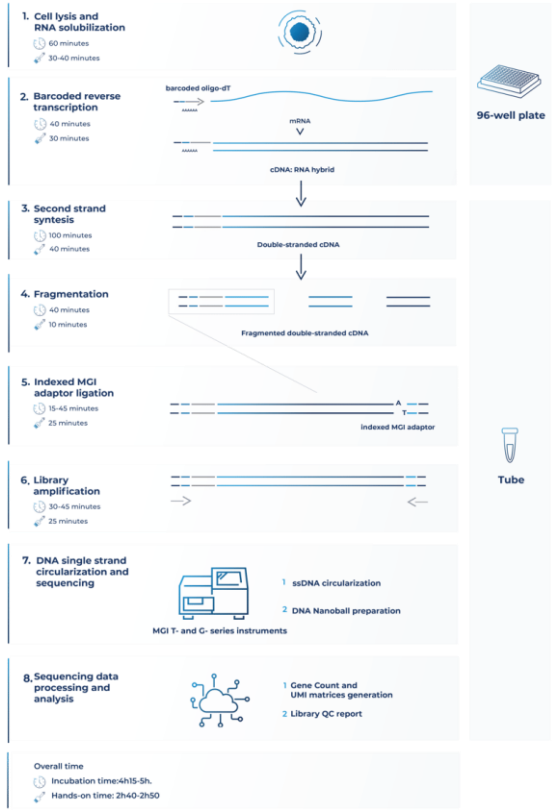
Hight throughput transcriptome analysis

- Whole transcriptome
- No RNA extraction required

BioSpyder temp-O-Seq (1 probe/transcript)



Alithea Mercury Drug-seq (3’)



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