

Variations in dietary preferences of gut-colonizing bifidobacteria

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Predictive Metabolic Profiling: *why and how*

Combine:

- Genome-based reconstructions of metabolic subsystems (biochemistry, transport, regulation), and
- Multi-omics datasets (abundances, transcriptomes, metabolomes)

to accurately predict:

- major metabolic phenotypes (nutrient requirements, utilization and biotransformation capabilities), and
- metabolic interactions (competition and cooperation)

in human gut microbiota to support:

- understanding its healthy versus impaired development, and
- rational selection of supplementary foods and probiotics for repairing microbiota immaturity

Gut microbiome metabolic economy

DIET

HOST

MICROBIOTA

Carbohydrates
(raw materials, energy)

Amino acids
(building blocks)

Vitamins/Cofactors
(micronutrients)

Prebiotics
Microbiota-directed foods

Digestive Health
Issues are Prevalent

DSM
Prebiotics are food for
beneficial bacteria*

CNS

Neuroactive metabolites
(Trp degradation)

Fermentation end-
products (SCFA)

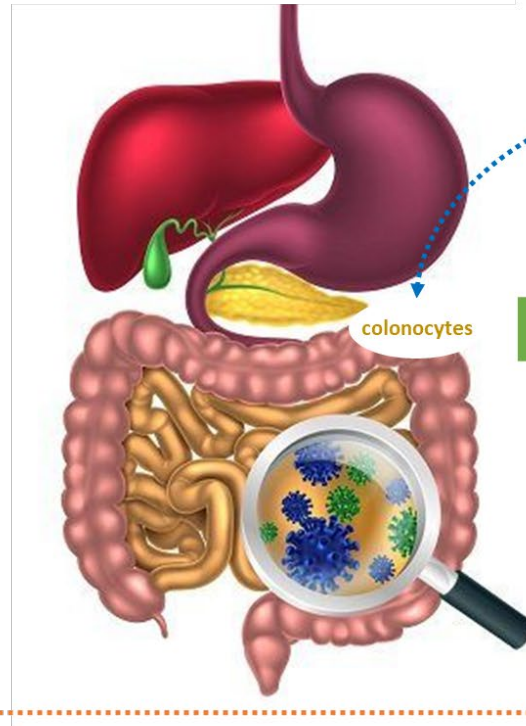
colonocytes

C

A

V

Metabolic exchange



PhenoBiome Approach

Objective:

Predict metabolic capabilities and interactions in microbial communities from genomic data

Approach:

[100+ metabolic phenotypes] x [1,000s gut microbes]

- Utilization of major nutrients (carbohydrates)
- Biosynthesis of essential nutrients (amino acids, B vitamins)
- Catabolic products (SCFA, tryptamine, etc.)

Methodology:

In silico metabolic reconstruction using bioinformatics tools and **mcSEED** genomic database

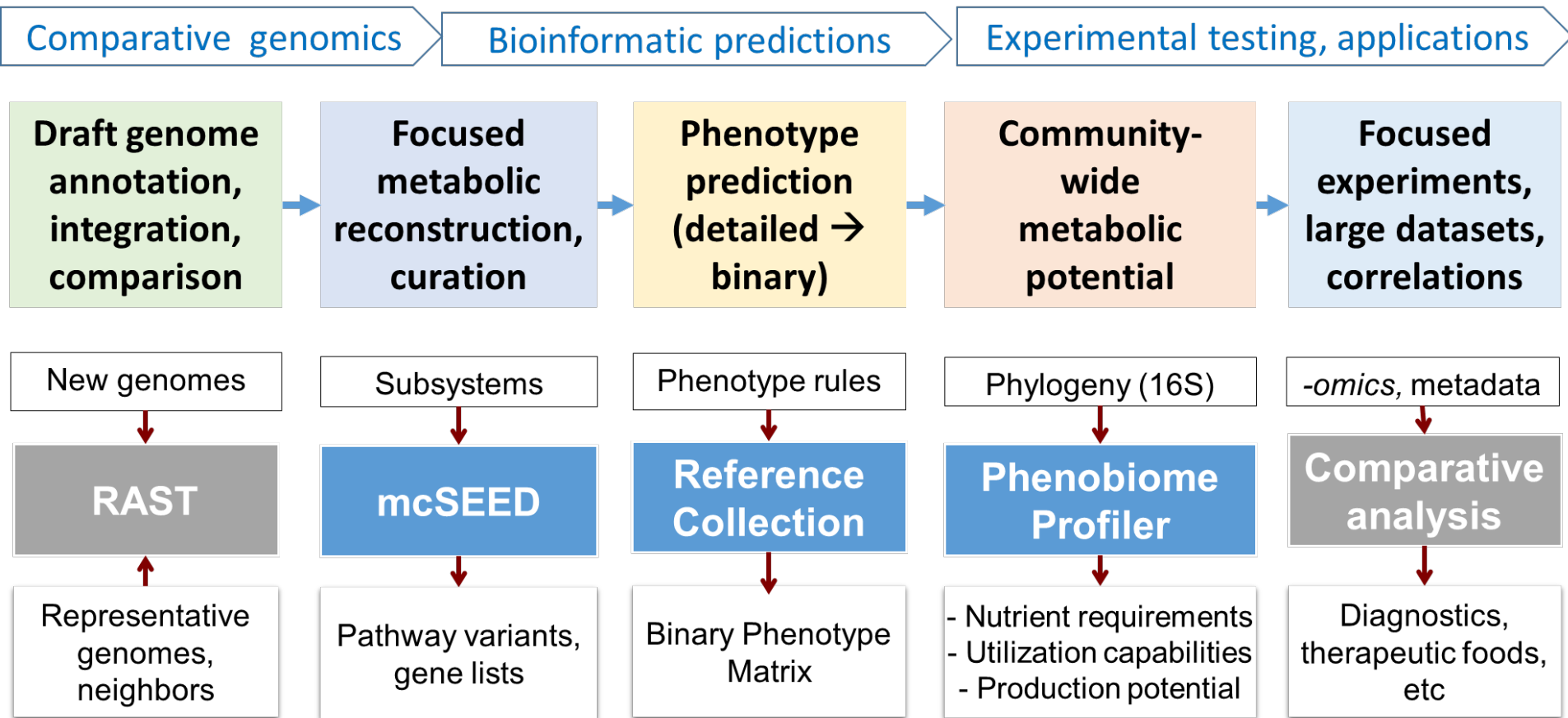
Applications:

Diagnostics, prevention and correction of disbiosis via rational nutrient supplementation

Binary Phenotype Matrix (BPM)

	Pheno type 1	Pheno type 2	Pheno type 3	Pheno type 4
Bug 1	1	0	1	1
Bug 2	0	1	1	0
Bug 3	1	0	0	0
Bug 4	0	1	0	1
Bug 5	1	1	1	1

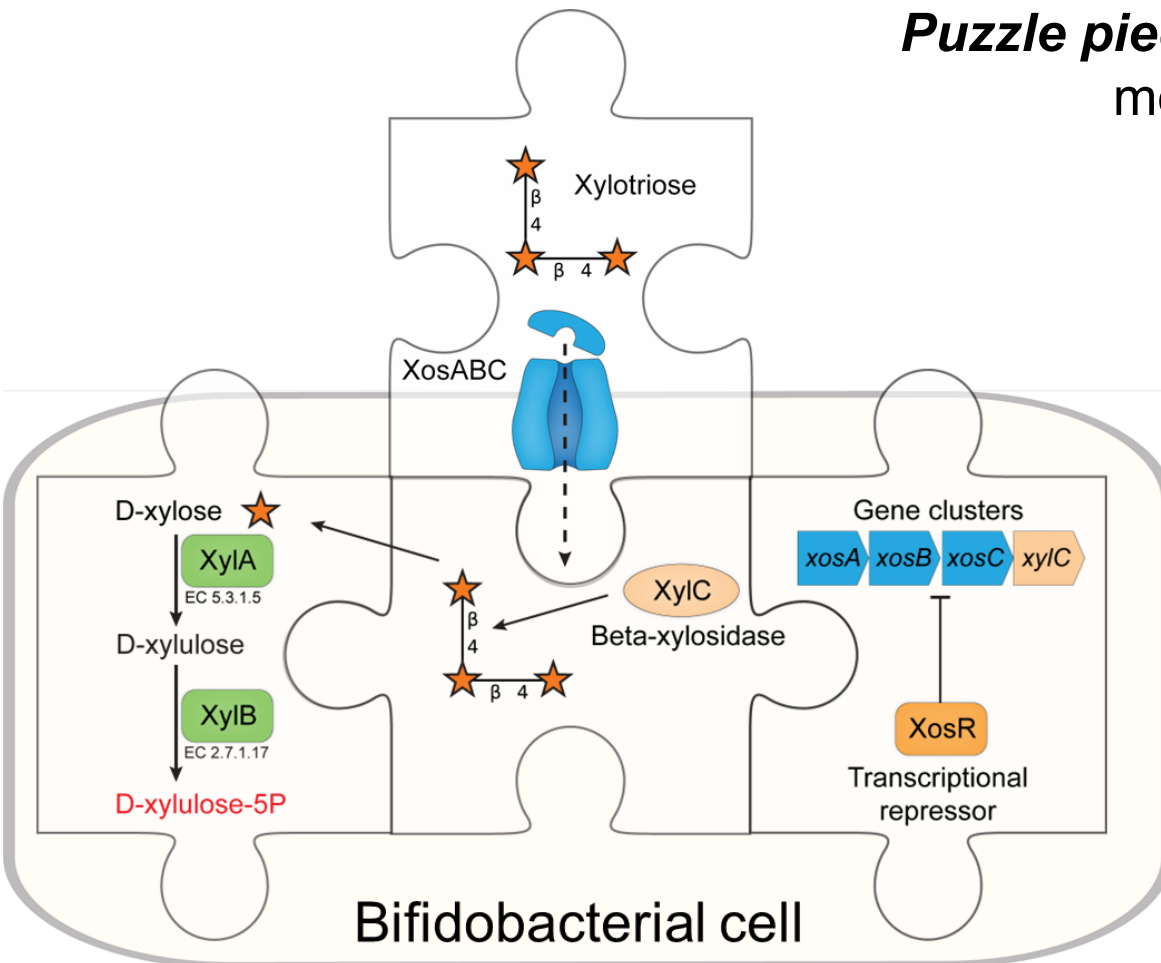
General Approach



Reconstruction of carbohydrate utilization

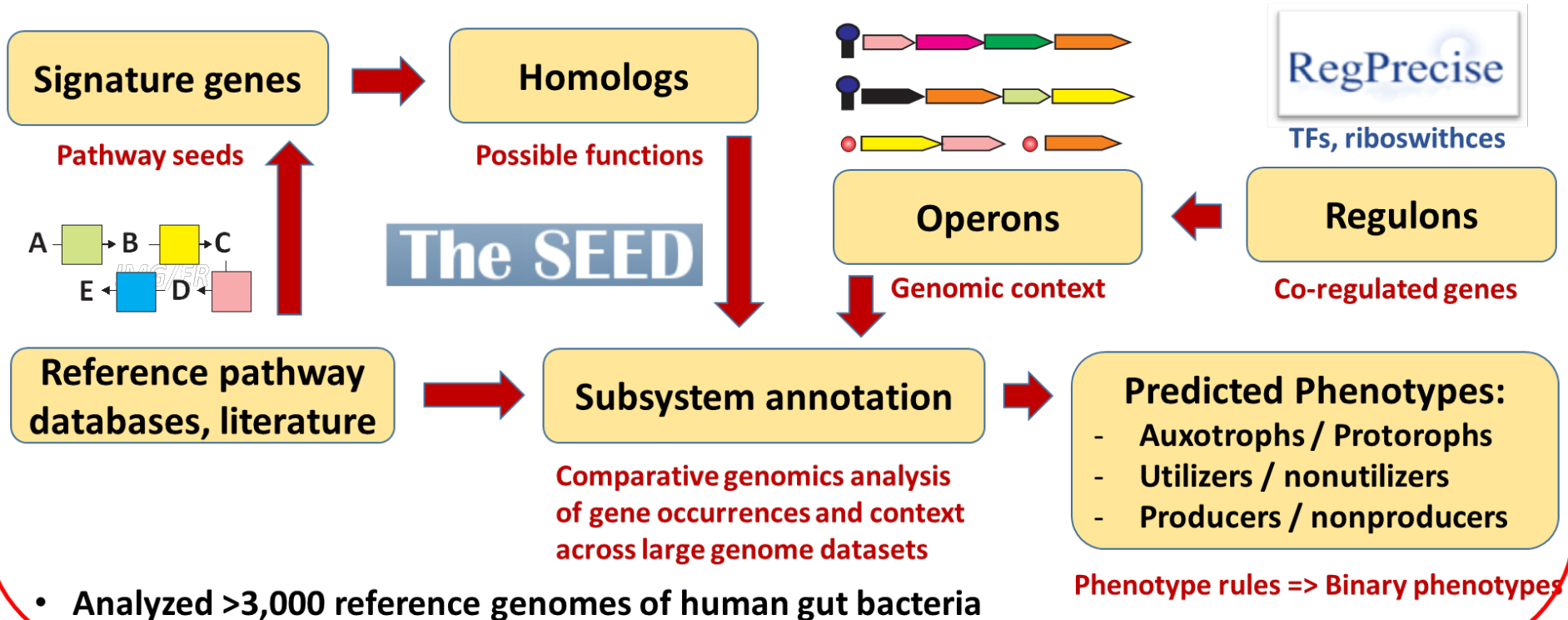
Puzzle pieces: functional elements of metabolic pathways

1. Glycan transporters
2. Glycoside hydrolases (GHs)
3. Downstream catabolic enzymes
4. Transcriptional regulators (-> regulons)

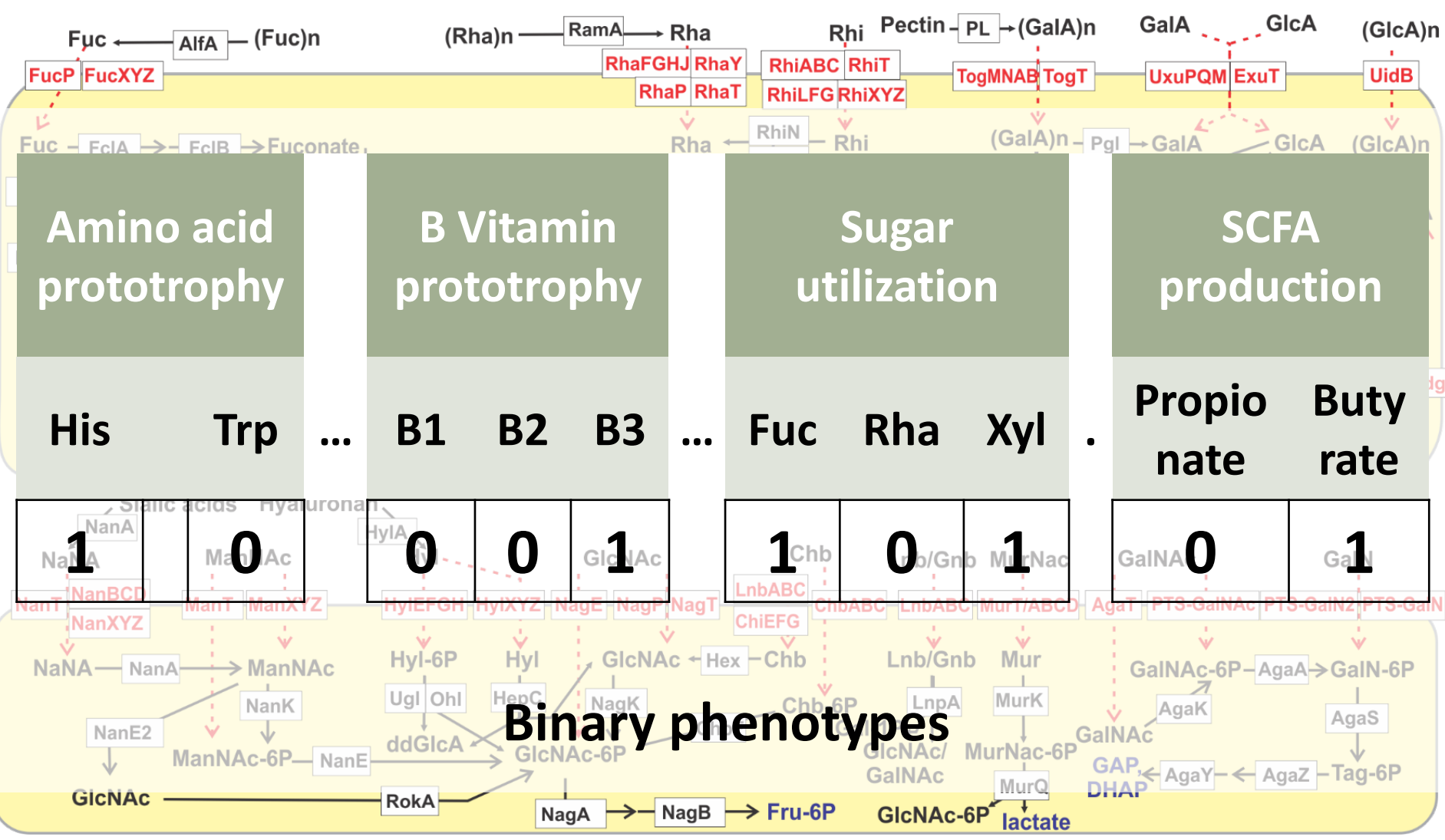


Curated reference set of metabolic pathways and phenotypes

- Comparative genomics reconstruction of metabolic pathways

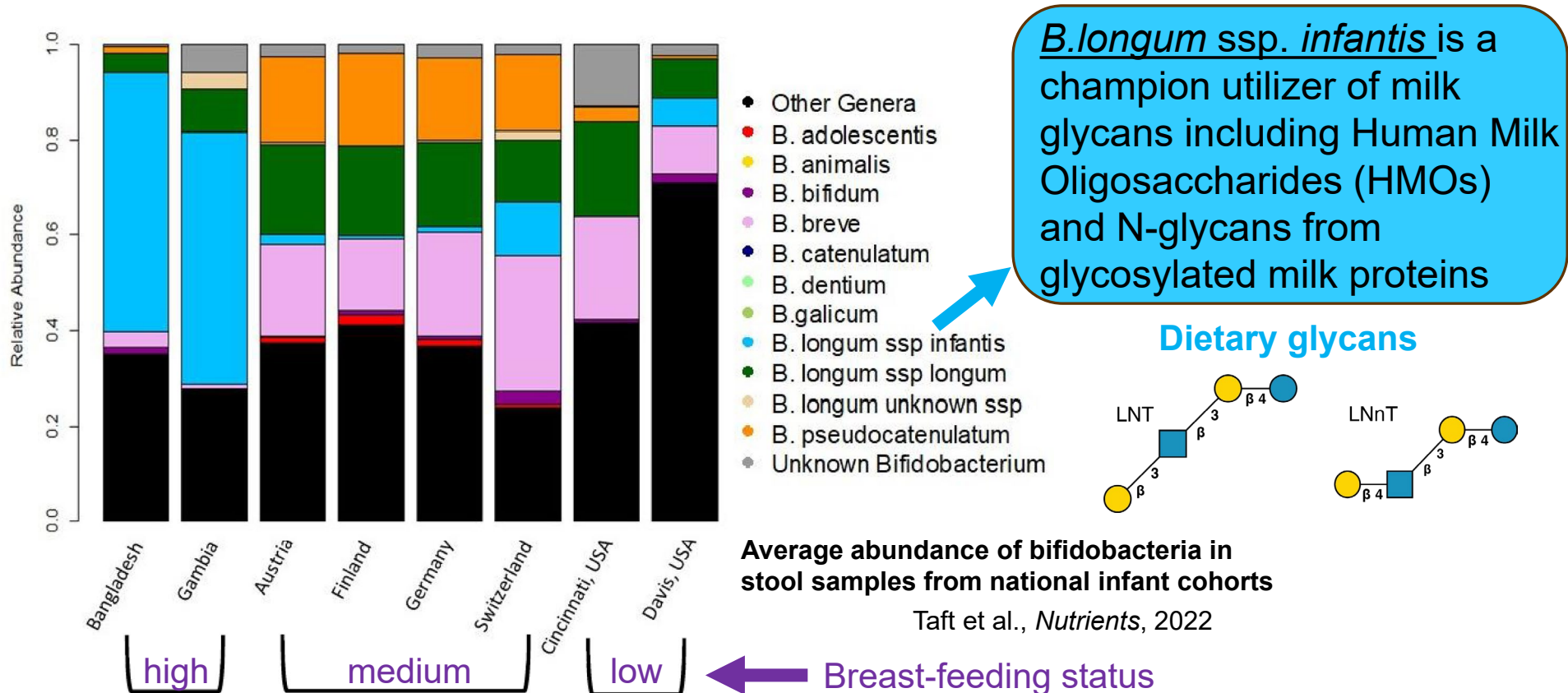


Metabolic reconstruction in 3,000s HGM reference genomes



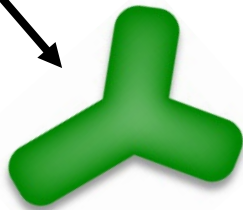
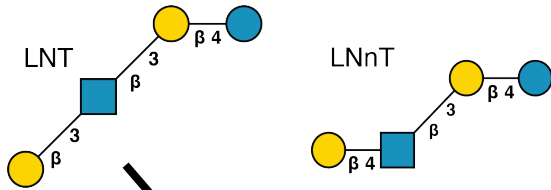
Bifidobacteria are early gut colonizers

- Most species are commensal and are considered beneficial to the host
- 16 species found in the human gut, including 3 subspecies of *B. longum*
- Dominant members of the breastfed infant gut microbiome



Why bifidobacteria are considered beneficial?

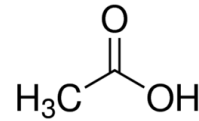
Dietary glycans:



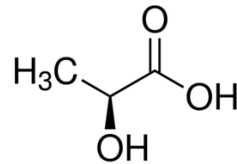
Carbohydrate metabolism

SCFAs:

Acetate

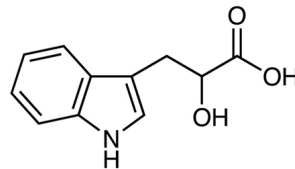


Lactate

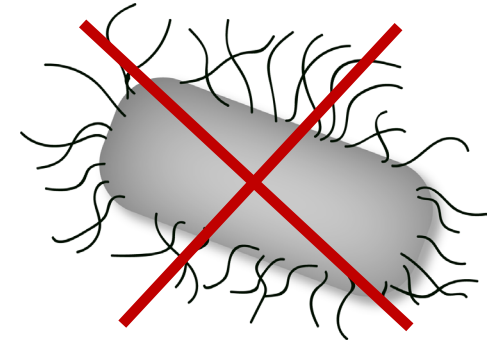


Amino acid metabolism

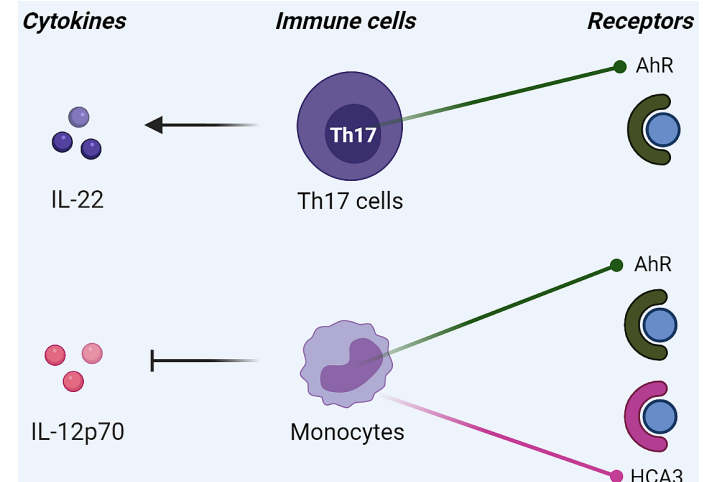
Indole-3-lactate



1. Decreased pH → protection from pathogens



2. Stimulation of anti-inflammatory immune response



- SCFAs cross-feeding to *Eubacterium*, *Faecalibacterium*
- IL-22 provides protection against gastrointestinal pathogens and promotes mucus production

Binary Phenotype Matrix (BPM) for bifidobacteria

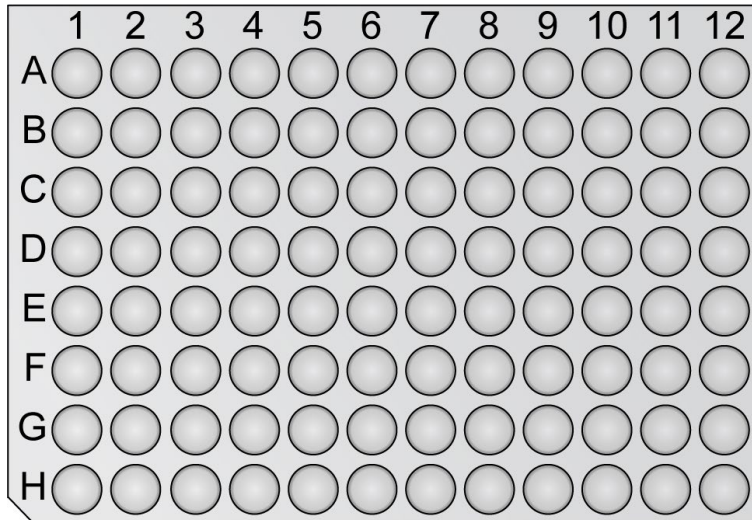
63 predicted carbohydrate utilization phenotypes

	Glucose	Raffinose	Inositol	Arabinose	Sorbitol	Lactose	LNT	Xylan	
360 genomes	<i>B. infantis</i> 1	1	1	0	0	0	1	1	0
	<i>B. infantis</i> 2	1	1	1	0	1	1	1	0
	<i>B. infantis</i> 3	1	1	0	0	0	1	1	0
	<i>B. longum</i> 1	1	1	0	1	0	1	1	0
	<i>B. longum</i> 2	1	1	0	1	0	1	1	0
	<i>B. longum</i> 3	1	1	0	1	1	1	0	0

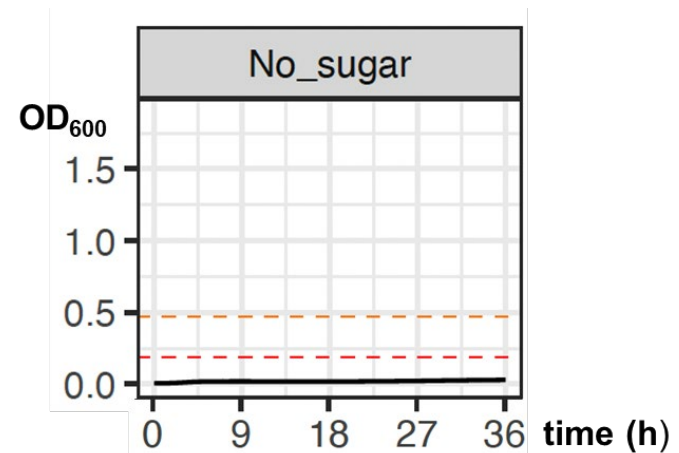
- 360 complete or high-quality draft genomes representing 13 species and 4 subspecies of *Bifidobacterium longum*

Experimental testing of predicted phenotypes

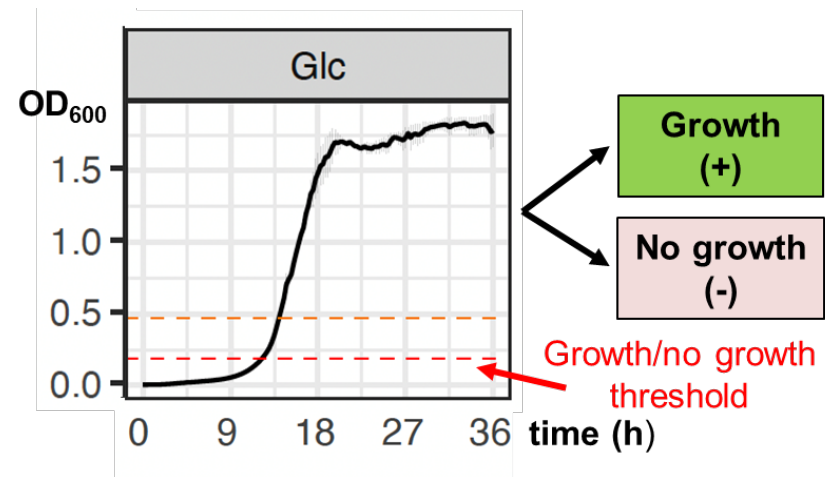
- **16 *Bifidobacterium* strains**
- **35 carbohydrates** (each used as a single carbon source)



In vitro growth experiments in anoxic conditions using a 96-well plate format



The basal medium does not support the growth unless a carbon source is added



Data are mean \pm SD (N=3)

Experimental testing: summary of results

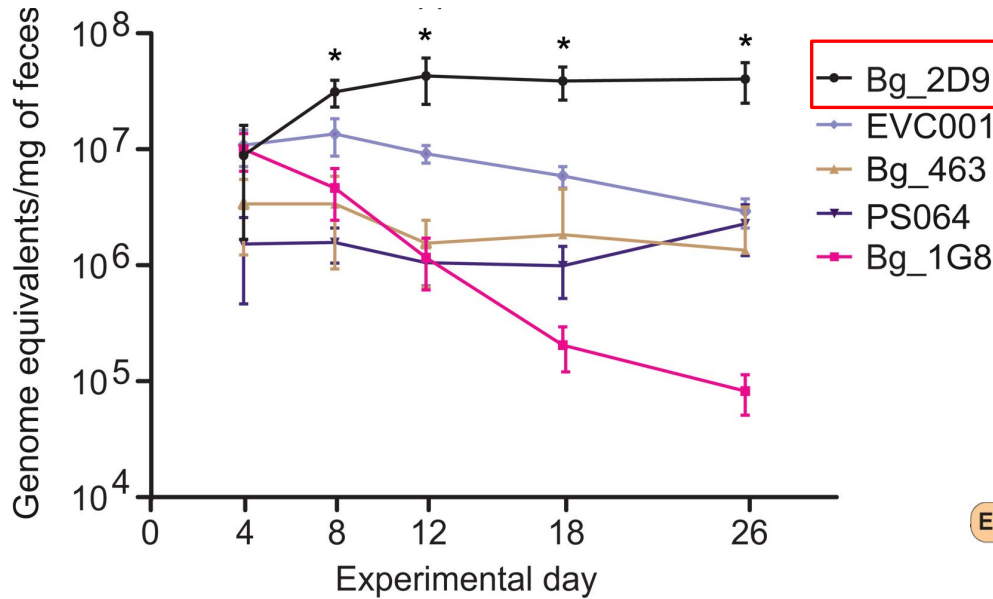
560 phenotypes in total: 16 strains x 35 carbohydrates

	Mono-saccharides	Di-/oligo-saccharides	Poly-saccharides	Other glycans	Total
Tested	208	240	48	64	560
Matched	175	233	46	63	517
Accuracy	84%	97%	96%	98%	92%
False positives	8	3	2	1	14
False negatives	25	4	0	0	29

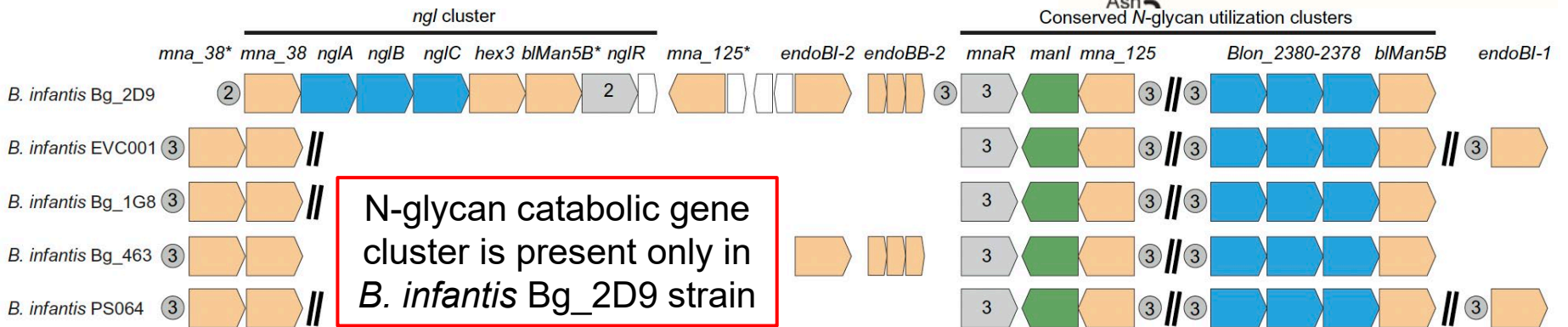
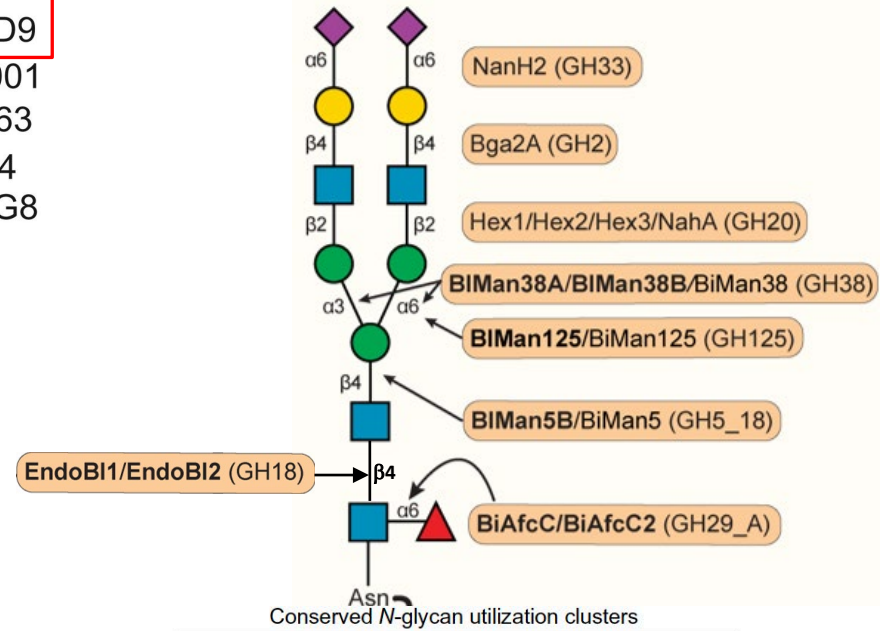
Overall, predicted phenotypes are in excellent agreement with growth data

Strain-level genomic variability affect fitness *in vivo*

Competitive fitness of 5 *B. infantis* strains in gnotobiotic mice



N-glycan structure and catabolic enzymes



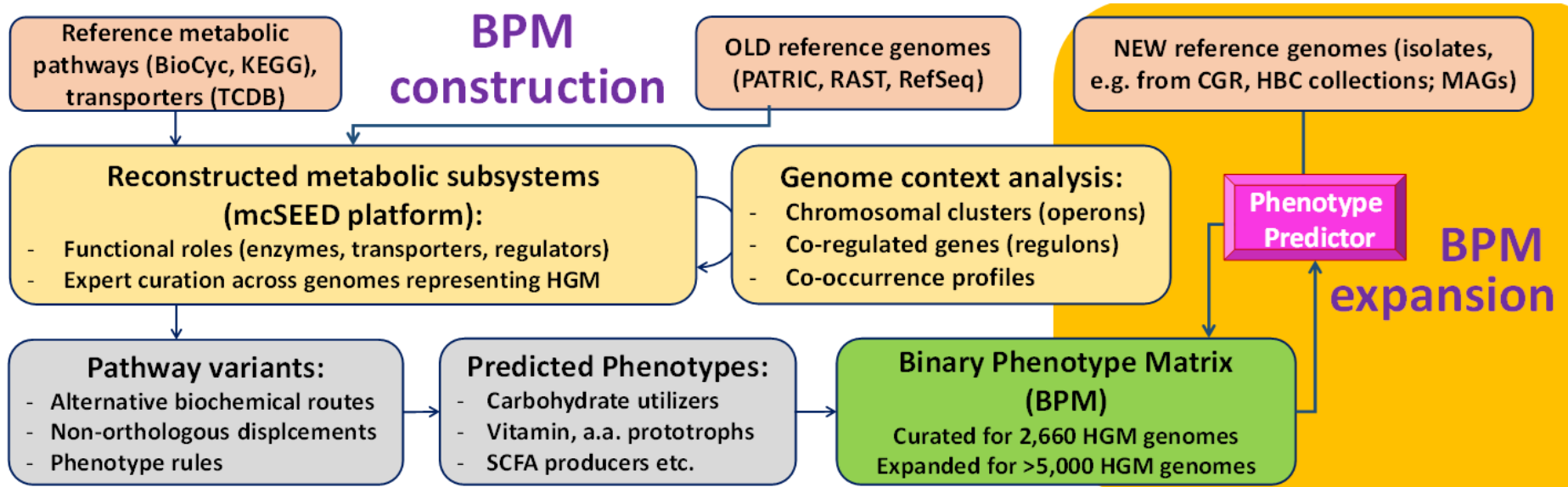
BPM propagation to expanded genomic collections

- New culture techniques -> expansion in numbers of microbial isolates for the **human gut**.
- **Metagenome Assembled Genomes** (MAGs) outnumber isolate genomes dramatically.

Challenge: How to use these increasing numbers of new genomes / MAGs ?

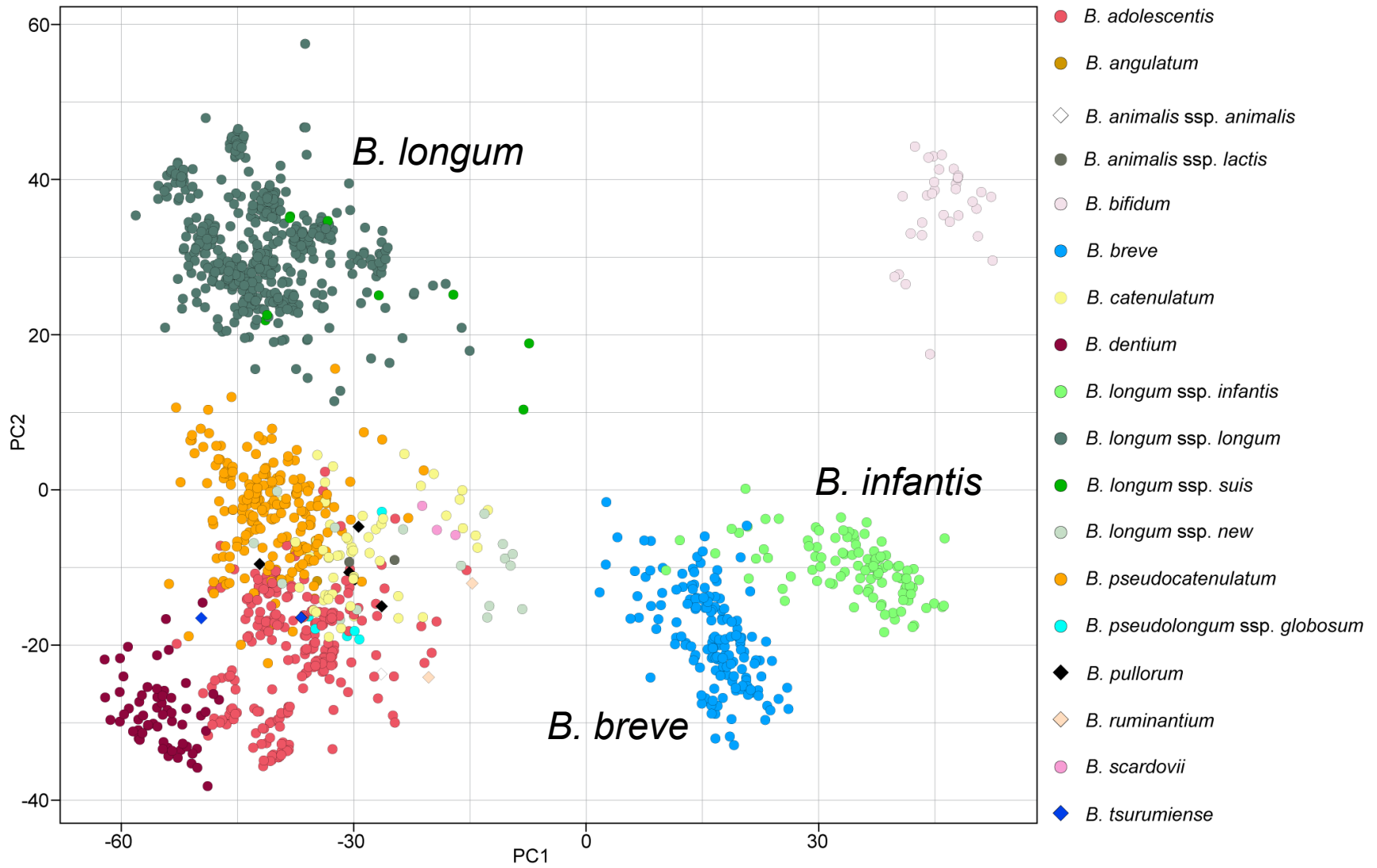
Phenotype Predictor

- A computational pipeline for automated propagation of curated metabolic phenotypes from the reference Binary Phenotype Matrix (BPM) to new genomes.
- Machine Learning *predictive models* for phenotype assignment with >99% accuracy.



- Applied to 300 isolate genomes & 2,400 MAGs to obtain expanded BPM for Bifidobacteria

Phenotype-based clusters of bifidobacteria

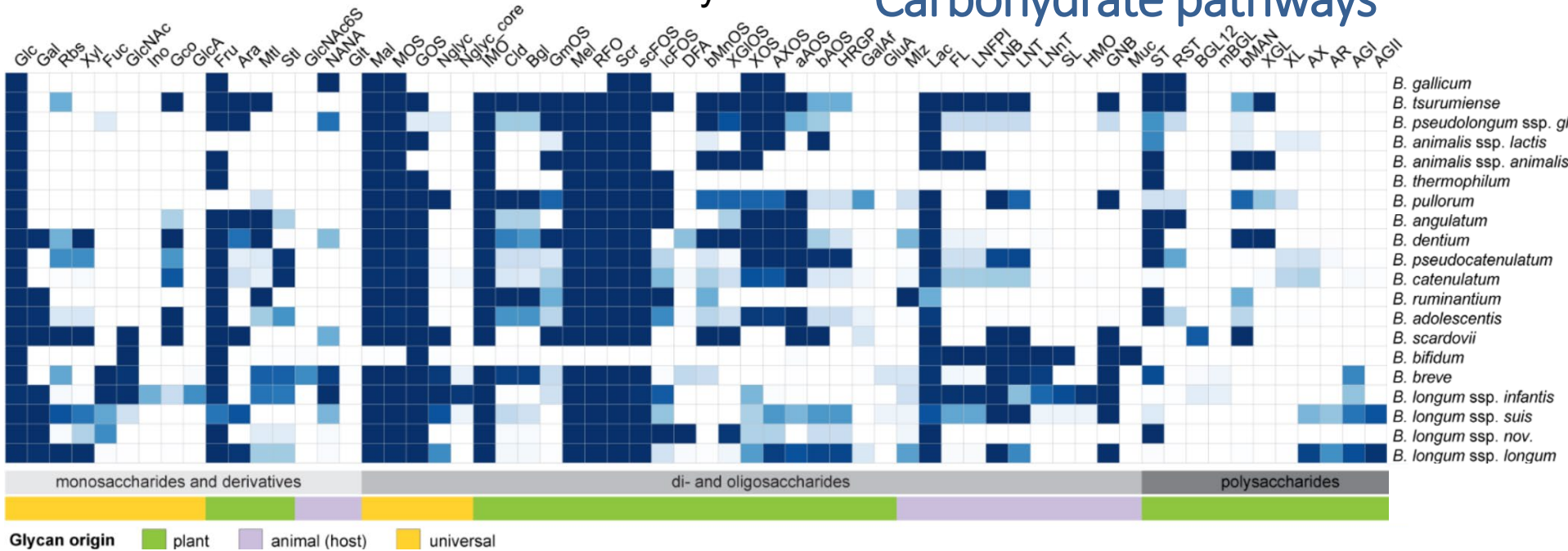


Logistic PCA of carbohydrate BPM (63 predicted metabolic pathways in 2,712 genomes)

Inter- and intra-species variability in bifidobacteria

- **Species-level:** some species are more equipped for *plant* glycan utilization, others prefer *host* glycans (HMO, N-glycans)
- **Strain-level:** substantial variability

Carbohydrate pathways



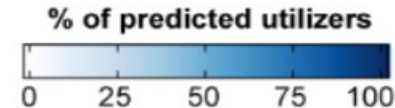
Most universally utilized glycans:

- Glucose - all species & strains
- Maltose, MOS, sucrose - 15 species (all strains)
- Fructose – 15 species, majority of strains
- Lactose - 14 species (all strains)
- Melibiose, raffinose - 14 species (all strains)

Heat map abbreviations:

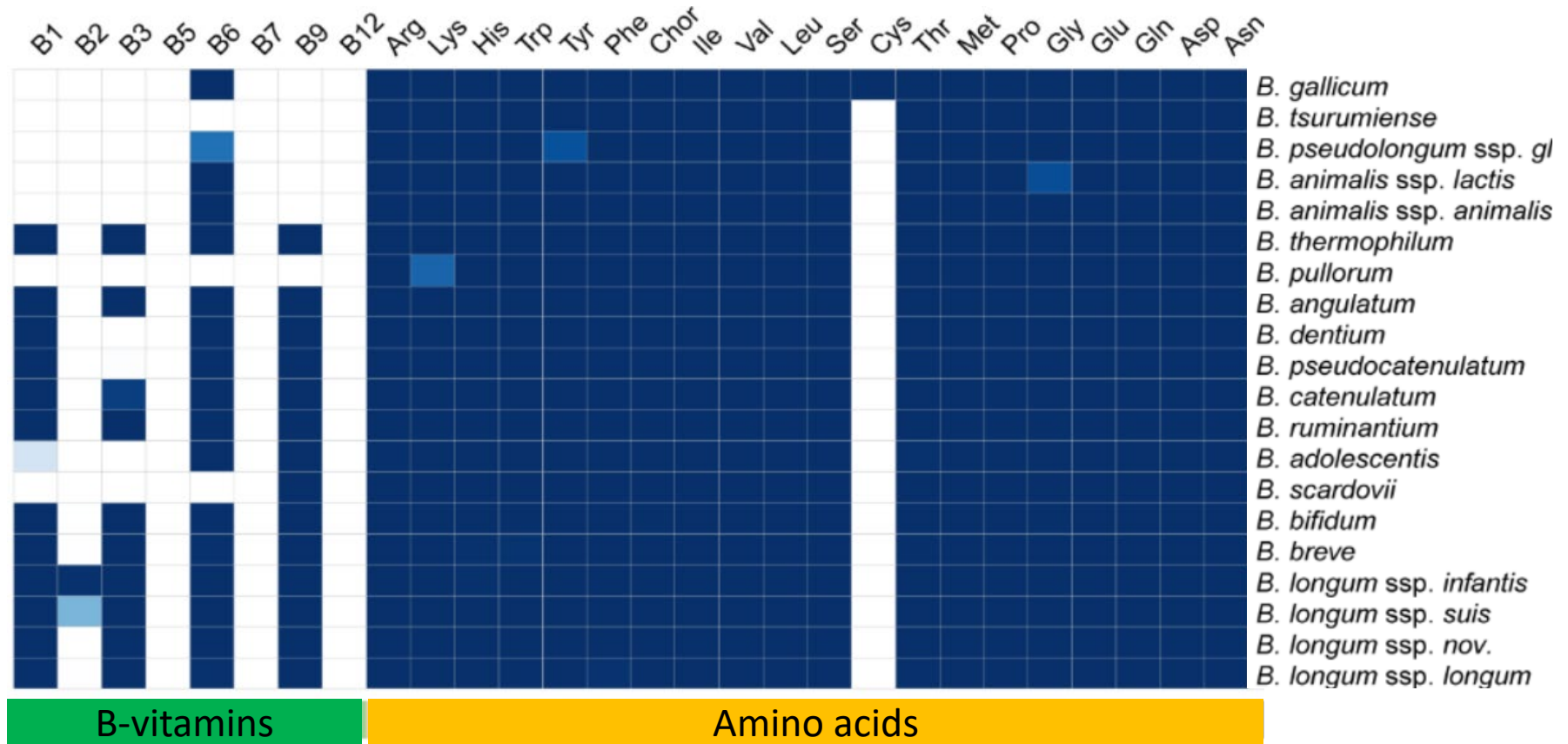
Dark blue: all strains are predicted **utilizers**

White: all strains are predicted **non-utilizers**

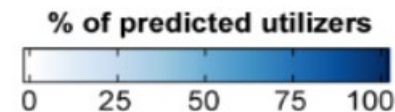


Inter- and intra-species variability in bifidobacteria

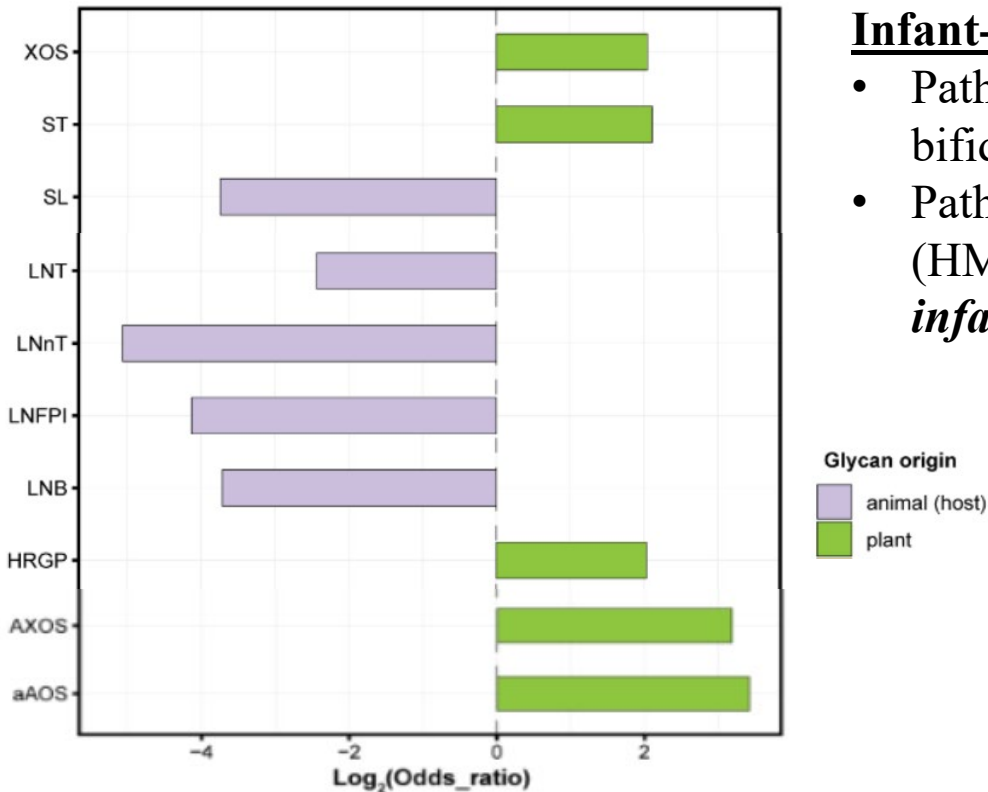
Synthesis of essential nutrients



Predicted Vitamin and Amino acid prototrophies are much more conserved compared to predicted Carbohydrate utilization phenotypes



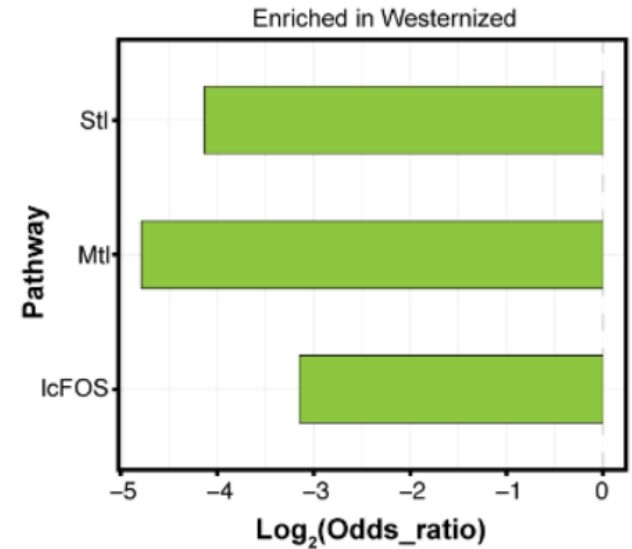
Pathway enrichment in bifidobacterial strains



Infant- vs. adult-associated strains:

- Pathways for plant glycans are enriched in bifidobacteria isolated from *adults*
- Pathways for Human Milk Oligosaccharides (HMO) and host N-glycans are enriched in *infant* strains

- Taxon-species enrichments associated with the lifestyle: pathways for sugar alcohol and long-chain fructooligosaccharides (FOS) utilization are enriched in *B. infantis* genomes from westernized samples compared to non-westernized strains



Conclusions and future perspective

- Genomics-based and knowledge-driven metabolic reconstruction of carbohydrate utilization pathways in 360 reference genomes of bifidobacteria
- Machine learning model for prediction of glycan utilization pathways in additional ~2,700 isolate and metagenome-assembled genomes
- Comprehensive understanding of how various bifidobacterial strains metabolize dietary and host glycans
- Substantial heterogeneity in the predicted glycan utilization capabilities, both at the species and strain levels
- Foundation for rationally selecting individual strains as probiotics and specific glycans as prebiotics

Acknowledgments

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