Mapping Gut Microbiota Interactions that are Robust to *C. difficile* Strain Variability and Nutrient Landscapes
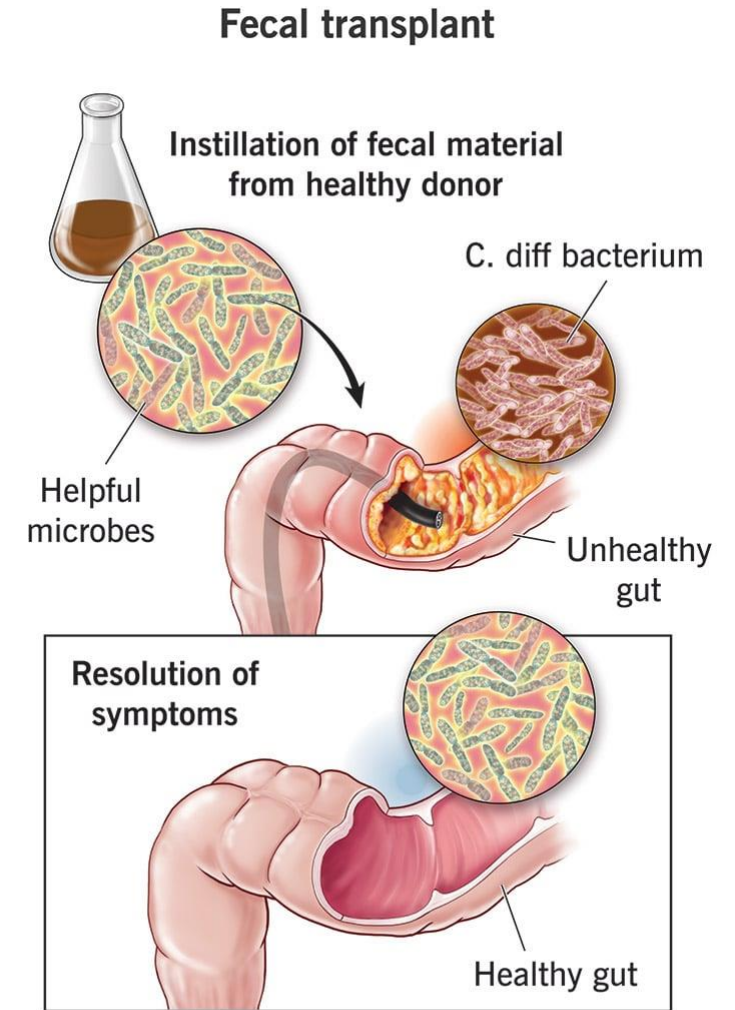
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6th International Conference on Microbiome Engineering

Current *C. difficile* treatments

- *Clostridioides difficile* is an opportunistic human gut pathogen.

- Antibiotics inhibit *C. difficile*, but they disrupt the commensal gut community that provides colonization resistance.

- Fecal Microbiota Transplant (FMT) is an attractive alternative, but **each FMT sample is bound to some level of uncertainty** in terms of efficacy and safety.

- FMT can also **unintentionally transfer antibiotic resistant bacteria** or even other pathogens.

Fecal transplant

Instillation of fecal material from healthy donor

C. diff bacterium

Helpful microbes

Unhealthy gut

Resolution of symptoms

Healthy gut

Cleveland Clinic ©2023

Lessa et al. (2015) *N. Engl. J. Med.,* 372:825-834

U.K. Health Security Agency (2022) *Clostridioides difficile* infection: updated guidance on management and treatment

Well-defined communities to inhibit *C. difficile*

- These problems could be overcome using **well-characterized microbial communities** that have been standardized and optimized to inhibit *C. difficile*.

- However, there are **variable successes in using defined consortia** to treat *C. difficile* infection in clinical trials.

Gut check: Seres Therapeutics shares plunge after microbiome drug fails in trial

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Berkeley Lovelace Jr.
@BERKELEYJR

SHARE f

Roger Pomerantz, Seres Therapeutics

Ratner (2016) *Nat. Biotech.*, 34:1004

Gap: Designing communities that are robust against *C. difficile* strain variation & nutrient environment

- *C. difficile* strains display extreme genetic variability and confront a changeable nutrient landscape in the gut.

- **Defined communities are less robust to environmental variability** than natural communities: Reduced richness, diversity, and functional redundancy.

- Previous studies that designed *C. difficile*-inhibiting consortia did not take into account **robustness towards strain variation and nutrient environment**.

FMT: >100 species   Defined communities: <10 species
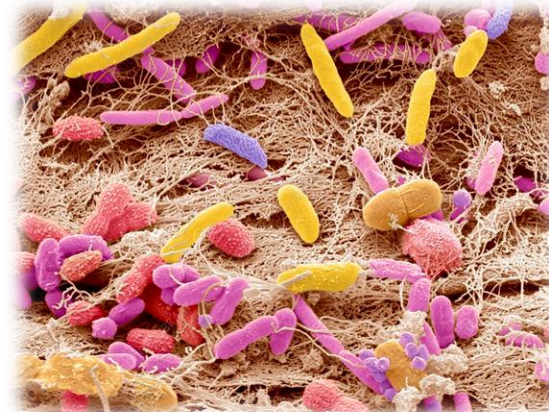
Elsas et al. (2012) *PNAS*, 109:1159-1164
Shade et al. (2012) *Front. Microbiol.*, 3:417

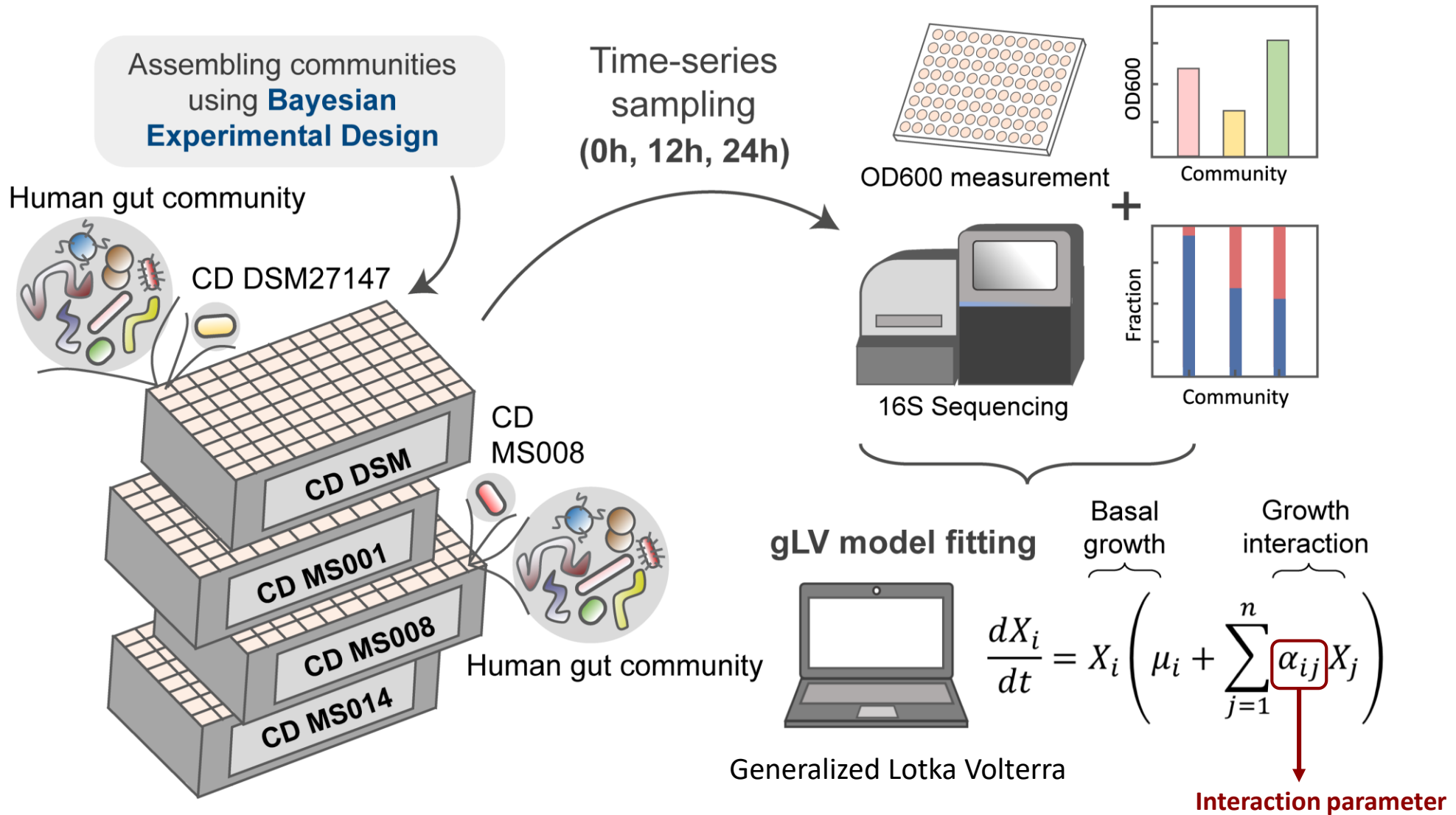# *C. difficile* strains possessed highly diverse genome
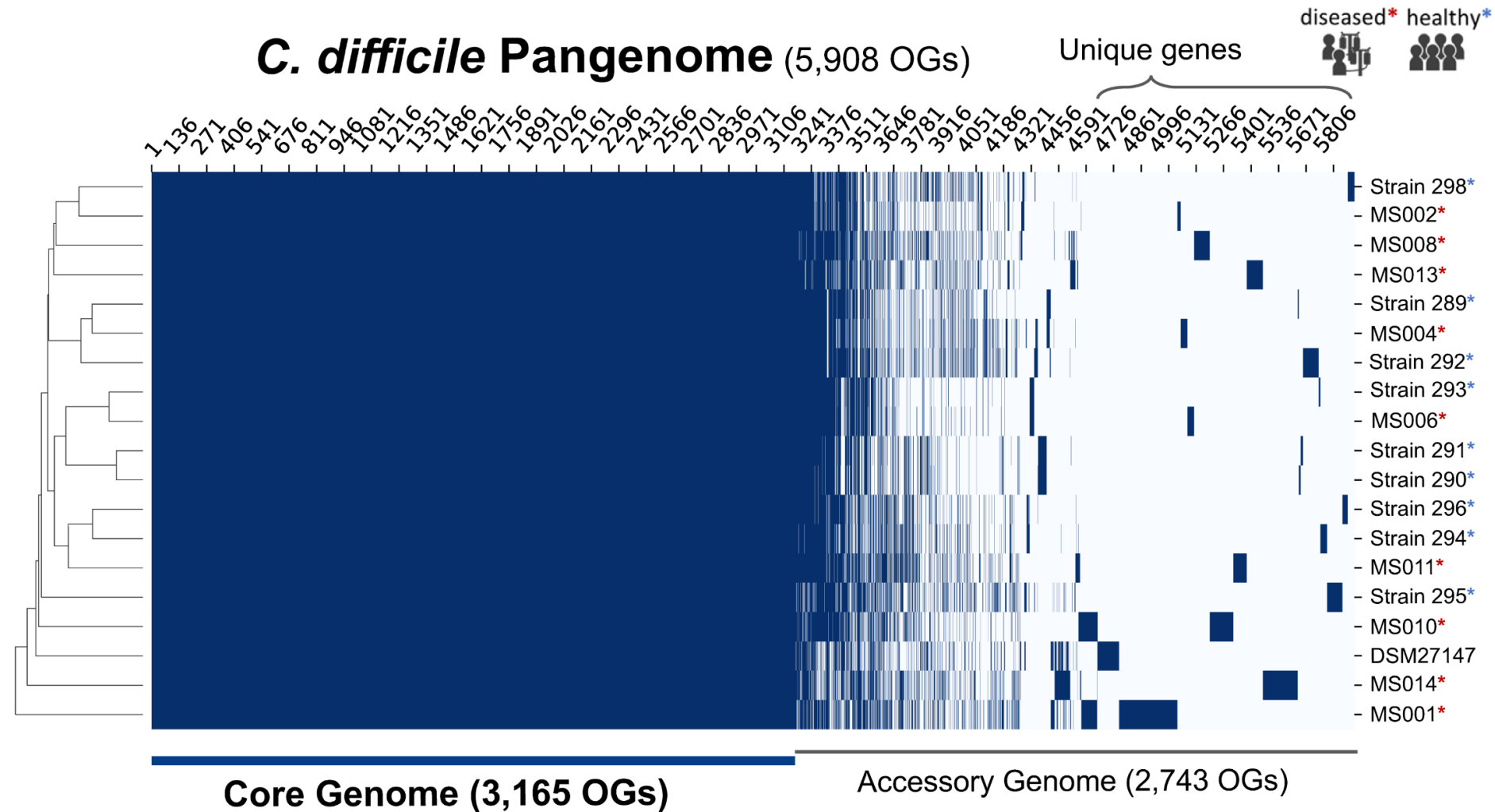
C. difficile strains have highly diverse genome with substantial variation in metabolic genes.

Extent of metabolic niche overlap between human gut species and *C. difficile*
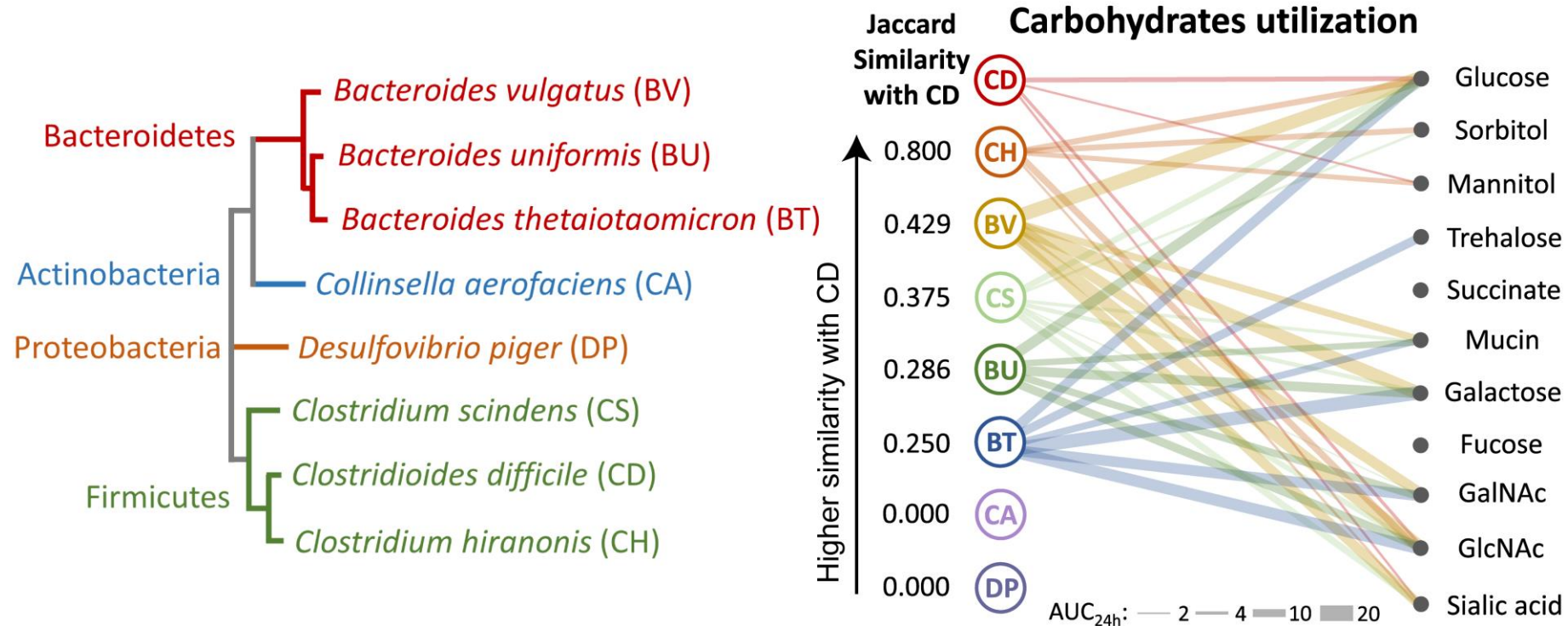
Based on monoculture growth profiles, **C. hiranonis has the largest niche overlap of carbohydrate utilization with C. difficile**. *C. hiranonis* can also utilize amino acids to perform Stickland metabolism, similar to *C. difficile*.
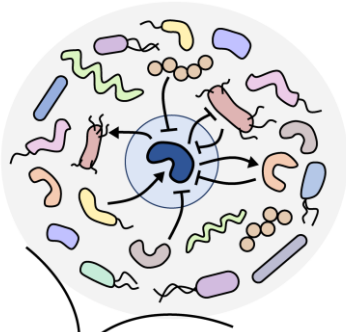
Workflow to study community interactions

$$\frac{dX_i}{dt} = X_i \left(\mu_i + \sum_{j=1}^{n} \alpha_{ij} X_j \right)$$

Generalized Lotka Volterra

Interaction parameter

Thompson et al. (2023) *PLoS Comp. Biol.*, 19:e1011436
Clark et al. (2021) *Nat. Commun.*, 12:3254
Venturelli et al. (2018) *Mol. Syst. Biol.*, 14:e8425

Human gut communities containing different *C. difficile* isolates display differences in interaction networks


High resource competition environment

Model
Glucose Media
High resource competition

Glucose

Communities with DSM

Communities with MS001

— positive
— negative

— 2.0
— 1.0
— 0.5

● 1.0
● 0.5

16%   84%

8%   92%

Communities with MS008

Communities with MS014

11%   89%

9%   91%
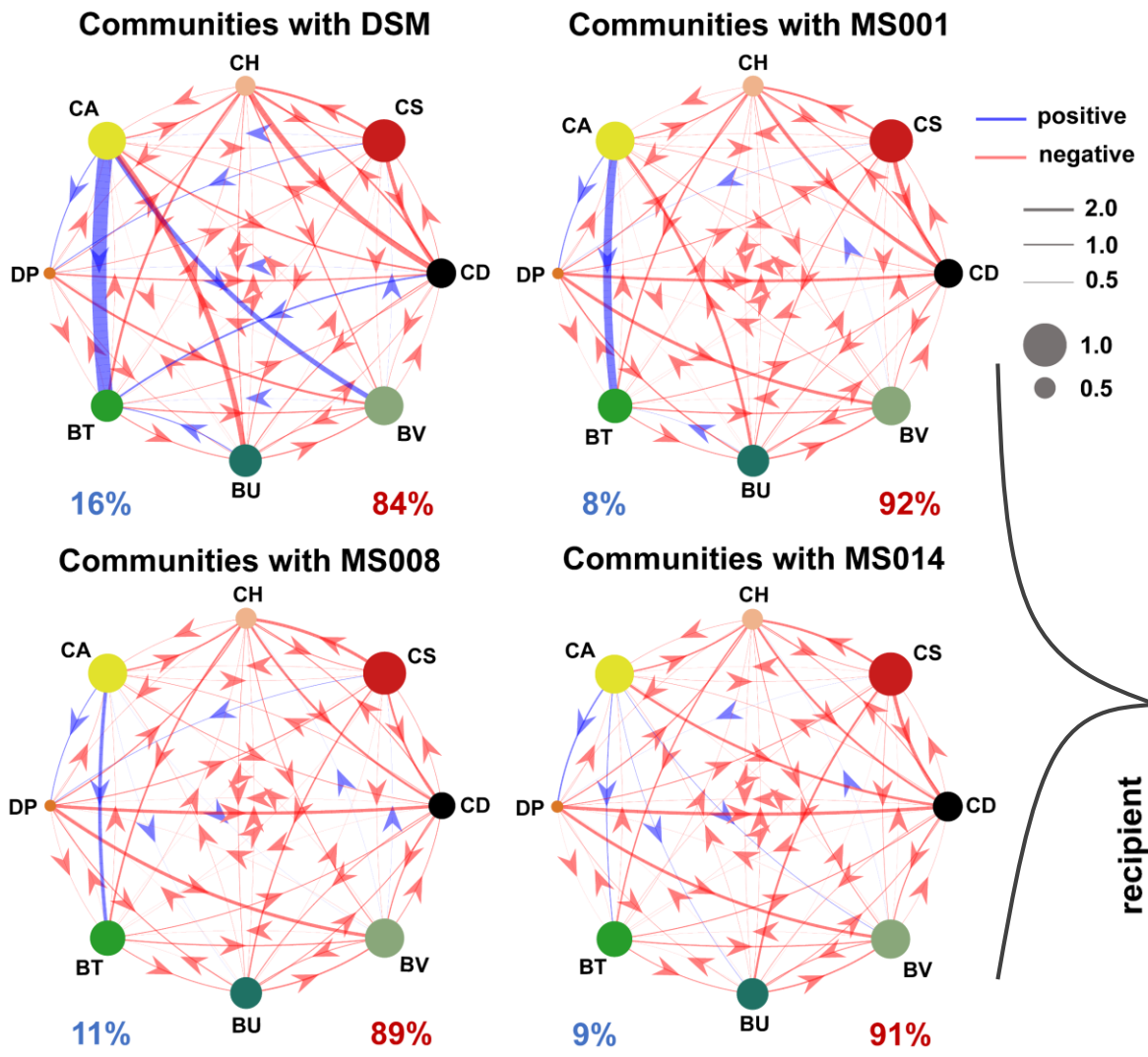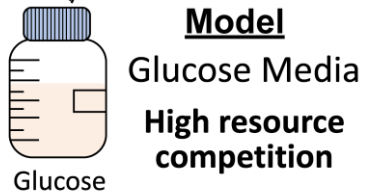
In the glucose media that represents high resource competition, the interaction networks in the communities are enriched with **negative interactions (84-92%)**, and **all gut species inhibit *C. difficile*.**
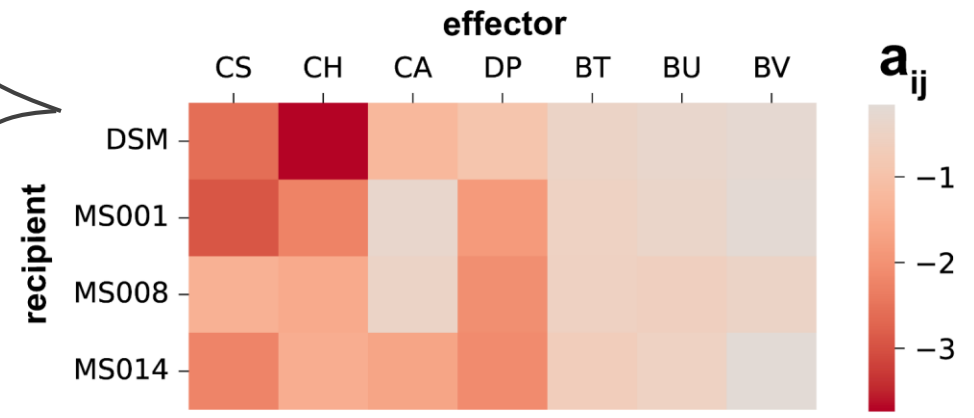
effector
a_{ij}

Human gut bacteria infrequently inhibit *C. difficile* in the presence of preferred carbohydrates
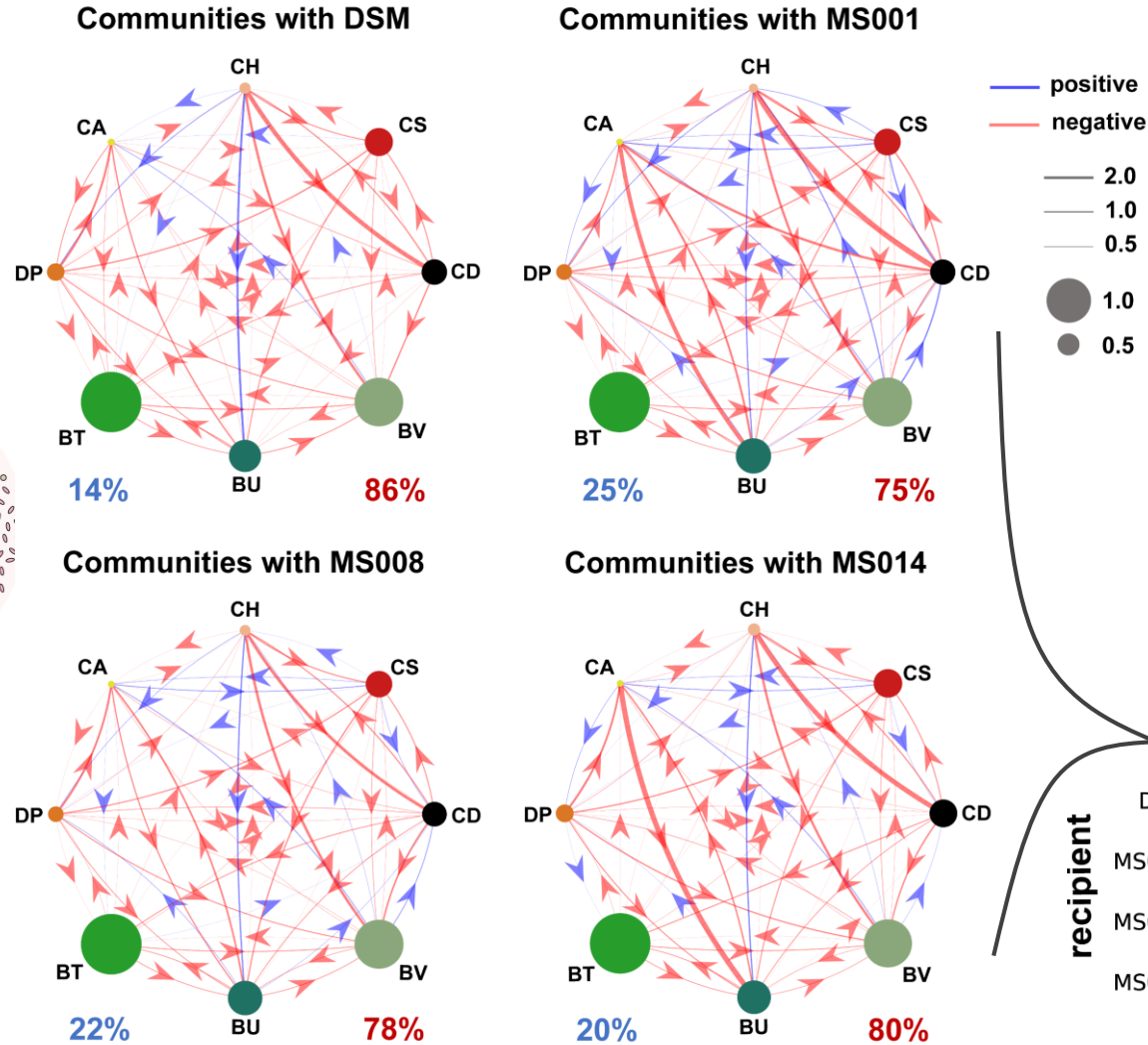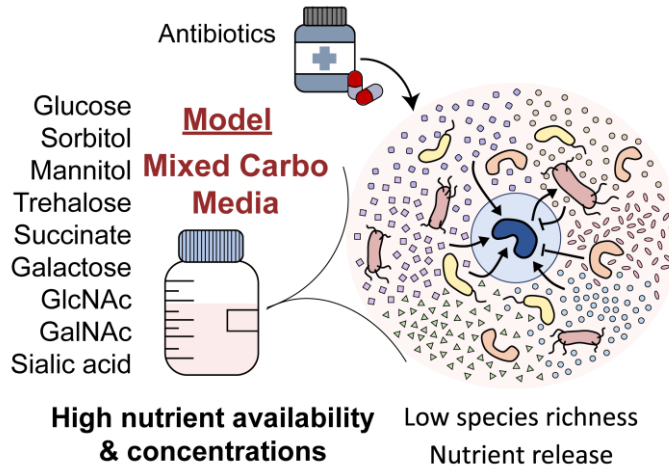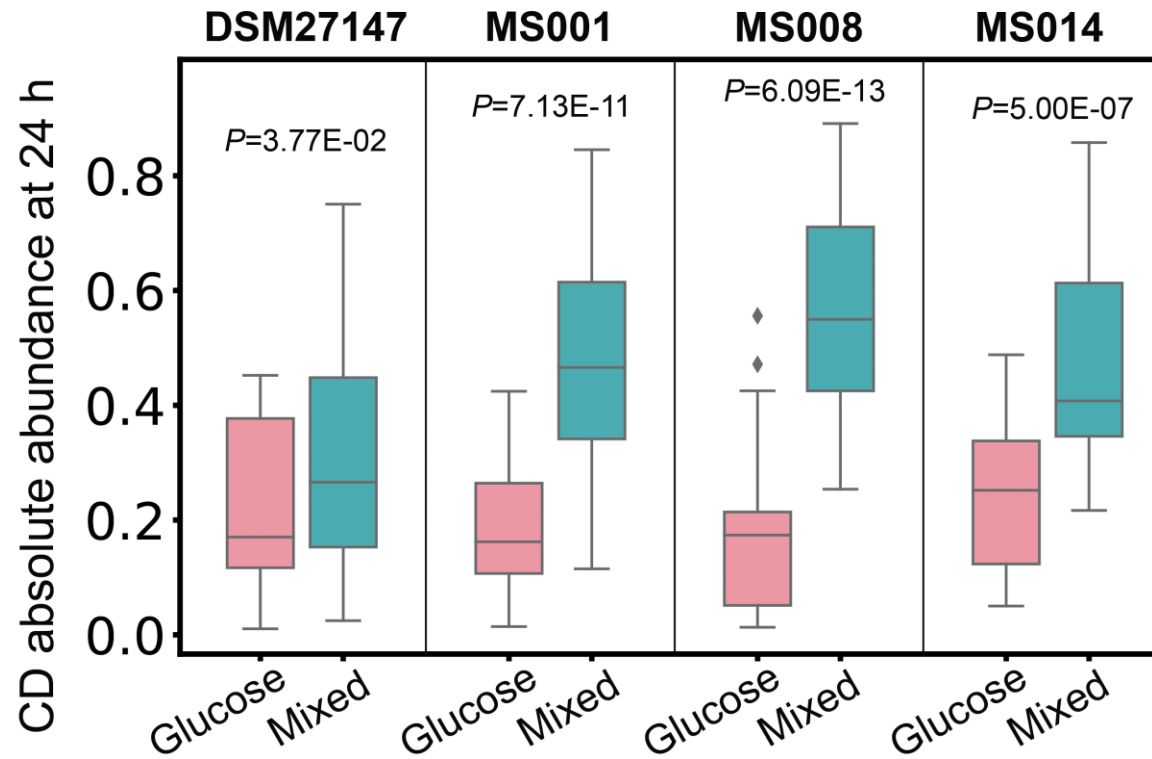

The interaction networks in the mixed carbo media display a **higher frequency of positive interactions**. Of 7 diverse human gut species, **only *C. hiranonis* displayed strong negative interactions** with each *C. difficile* strain.
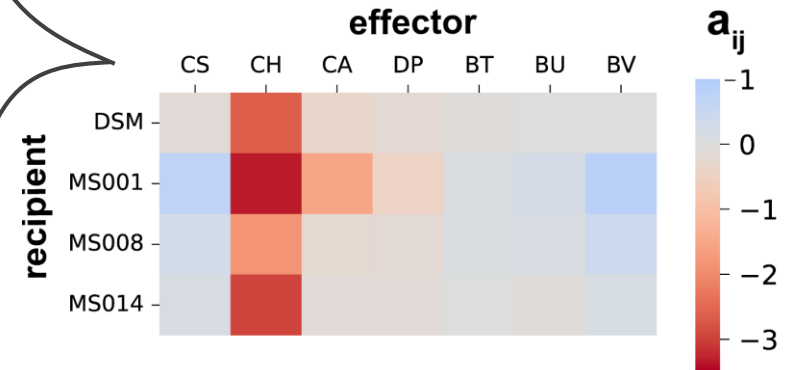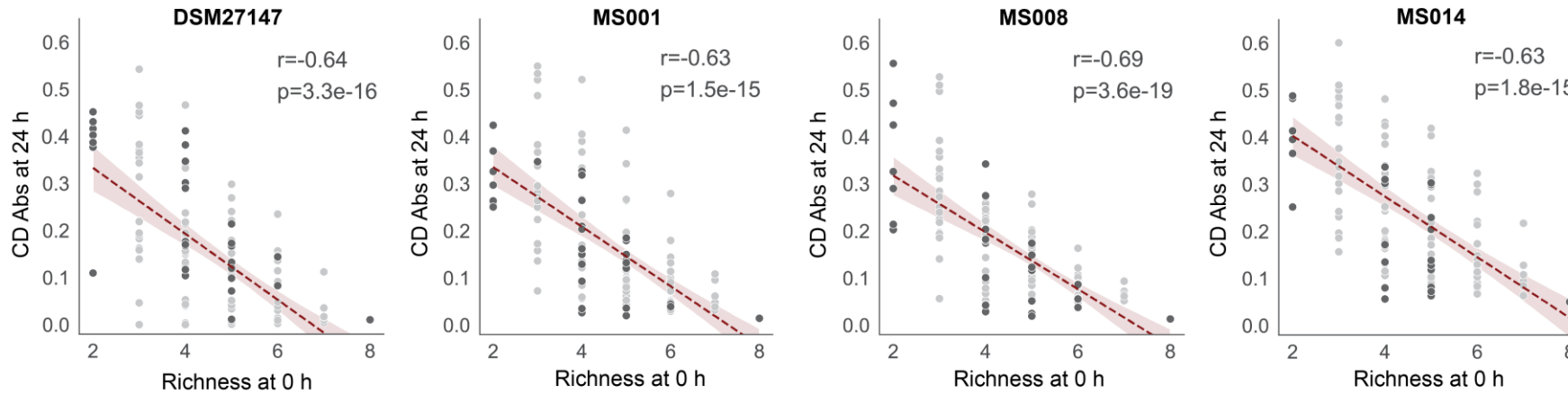
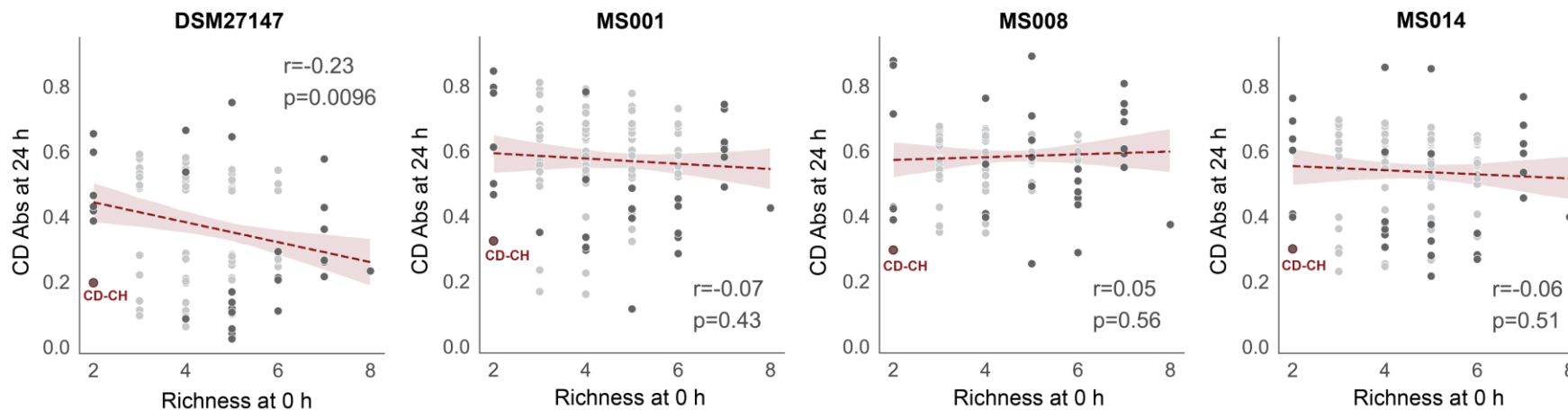# *C. difficile* growth in the Mixed Carbo Media vs. Glucose Media

The abundance of all *C. difficile* strains in communities was higher in the mixed carbo media.

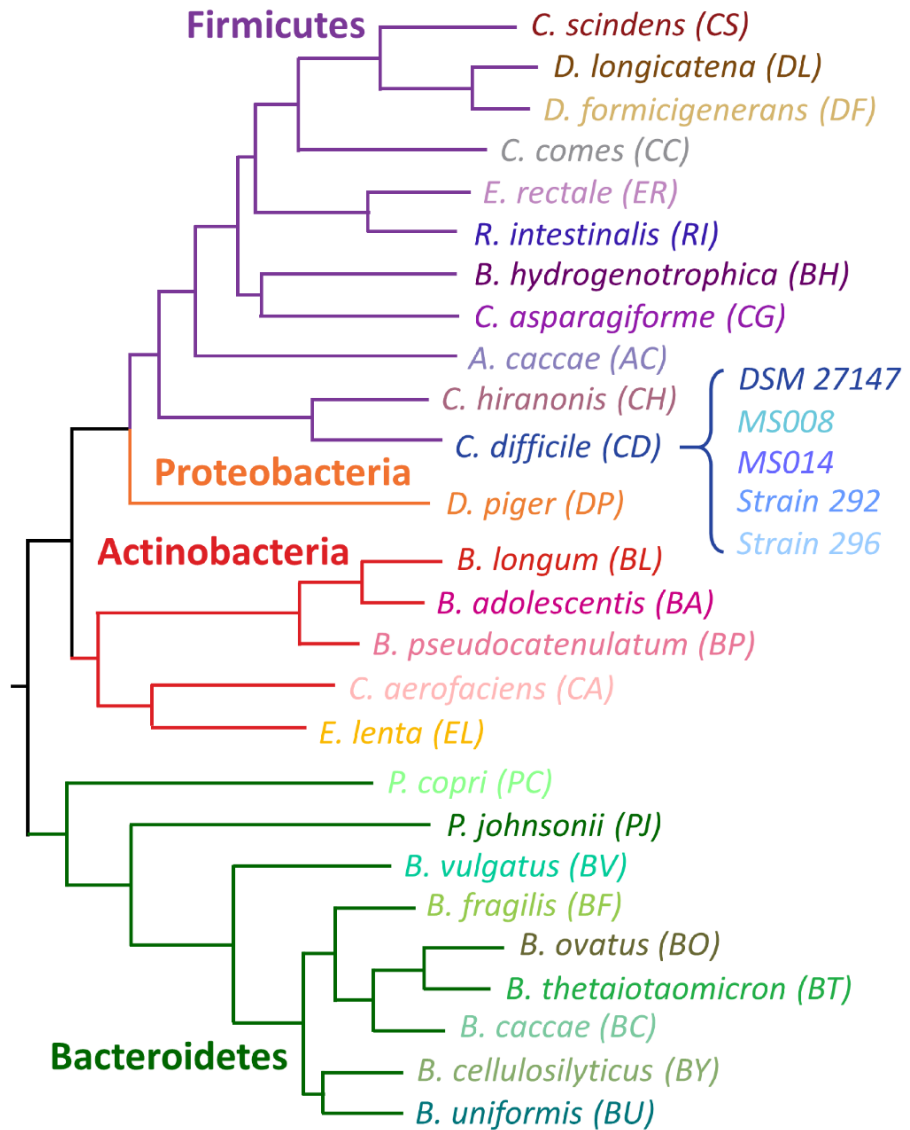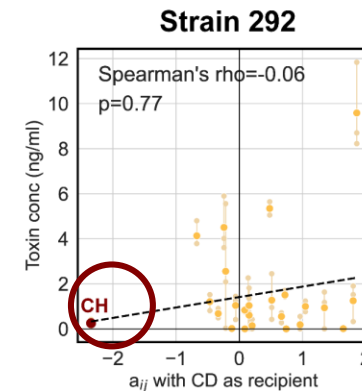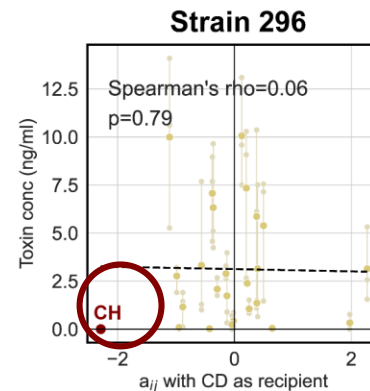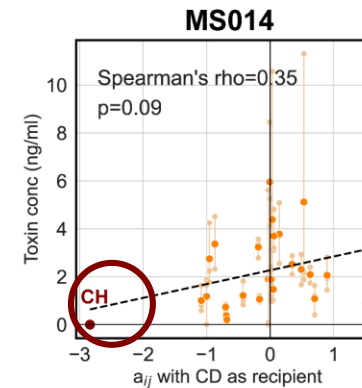# *C. difficile* growth in the Mixed Carbo Media vs. Glucose Media
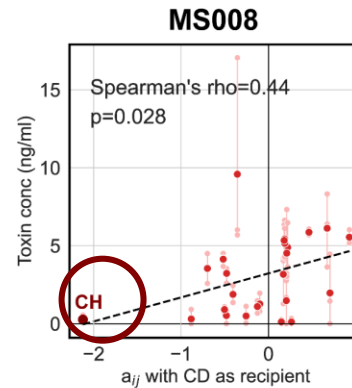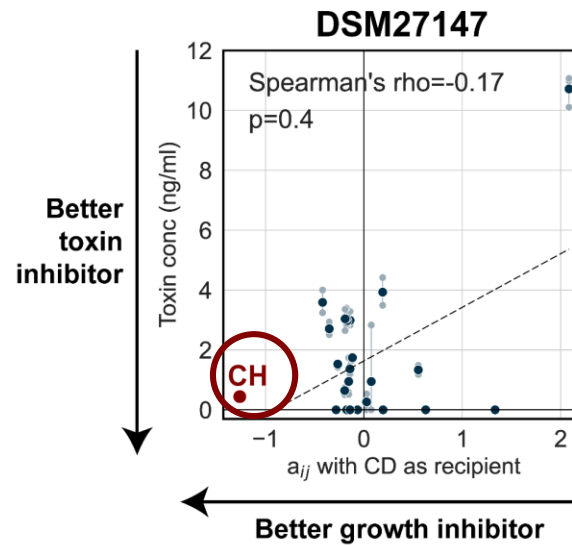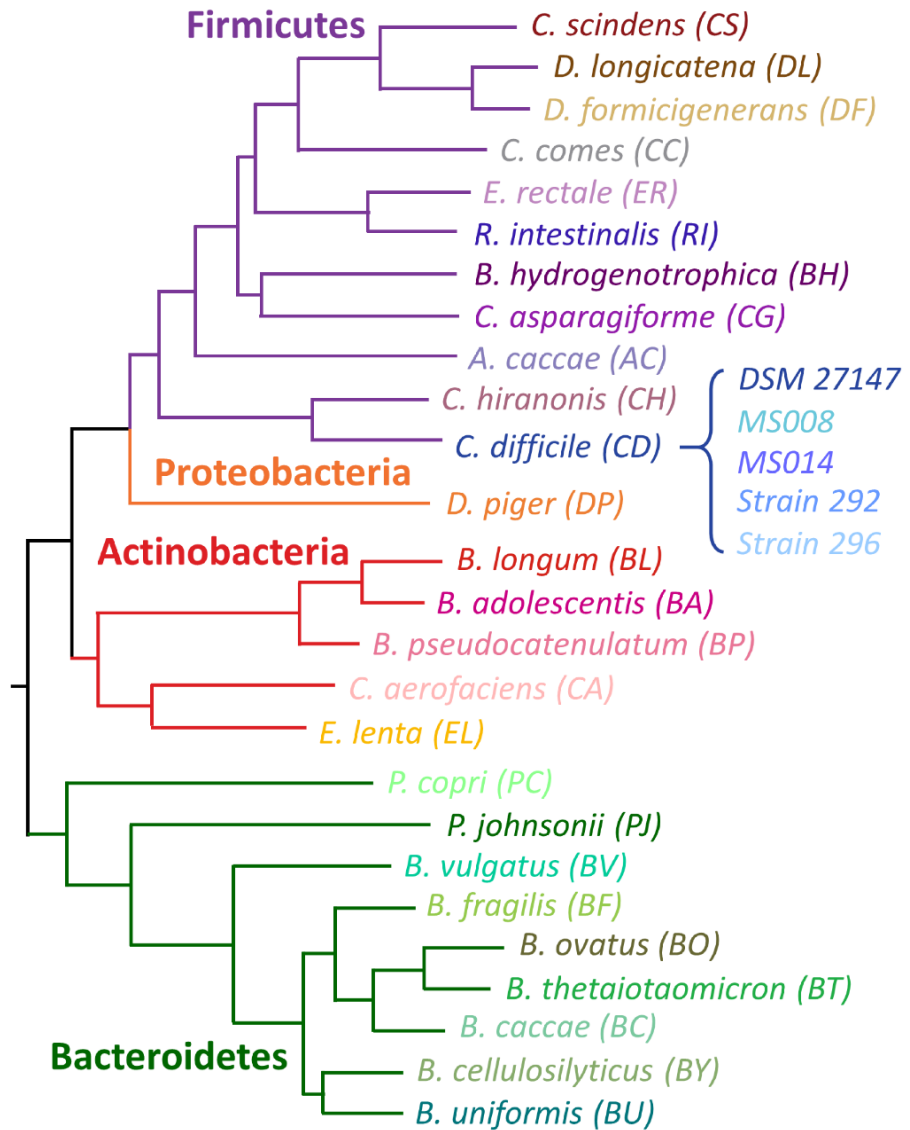

There's a strong negative dependence between *C. difficile* growth and species richness, but this is **much less apparent and even non-existent in the media with abundant resources for *C. difficile* to consume.**

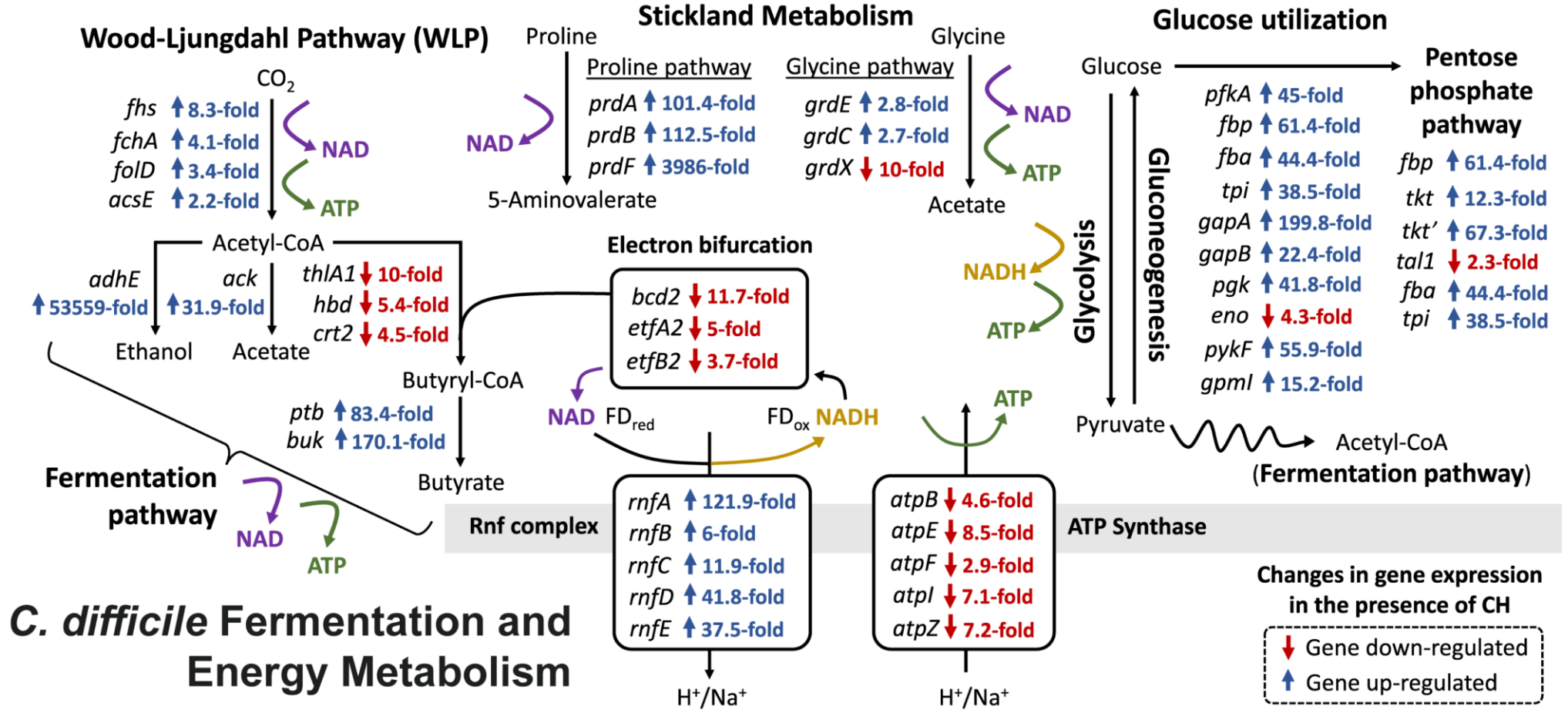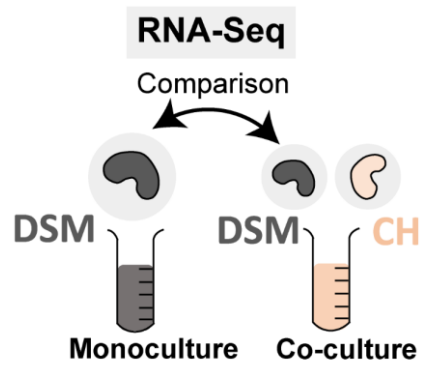**Profiling *C. difficile* toxin production in communities**

Profiling *C. difficile* toxin production in communities

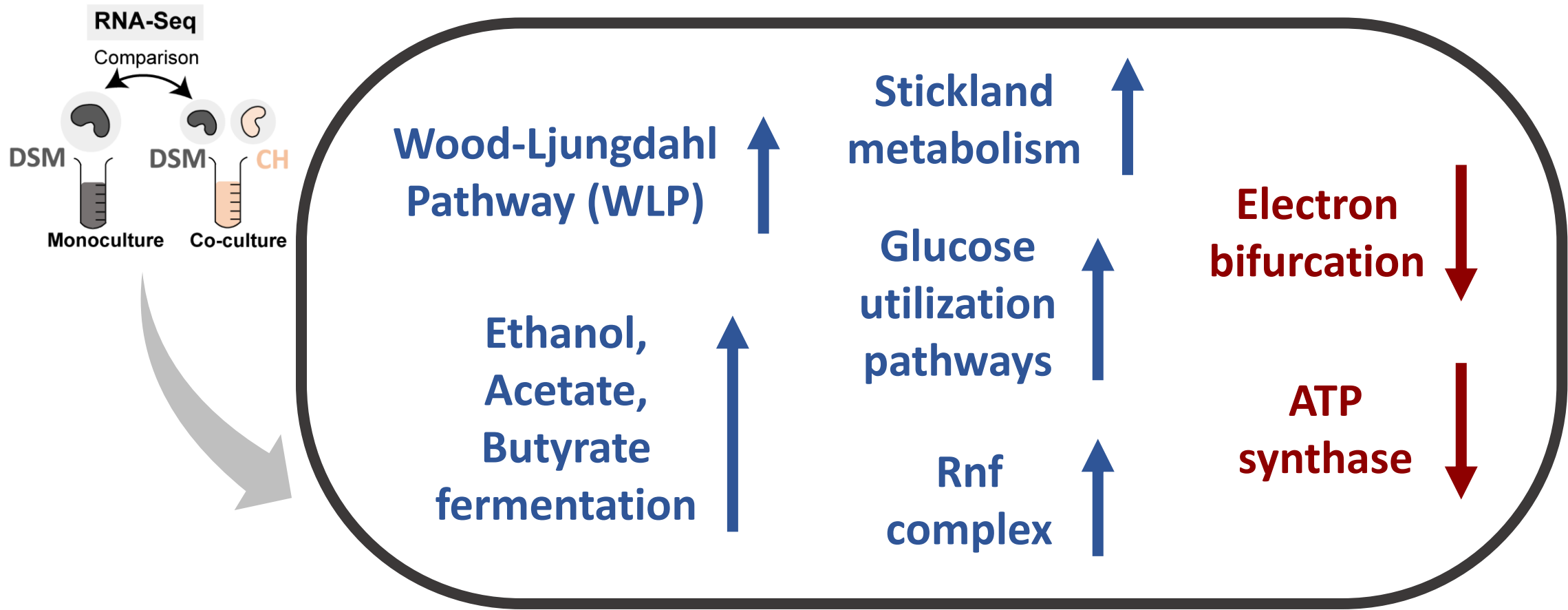Toxin production **is not correlated** with the inferred inter-species growth interactions.

C. hiranonis is the only species that **robustly inhibited both *C. difficile* growth and toxin production** of diverse *C. difficile* strains.

# *C. hiranonis* massively altered *C. difficile* metabolism and toxin production

RNA-Seq

Comparison

DSM ⟷ DSM | CH

Monoculture | Co-culture

Wood-Ljungdahl Pathway (WLP)

CO_2

fhs ↑ 8.3-fold
fchA ↑ 4.1-fold
folD ↑ 3.4-fold
acsE ↑ 2.2-fold

→ NAD
→ ATP

Acetyl-CoA

adhE ↑ 53559-fold | *ack* ↑ 31.9-fold

Ethanol | Acetate

thlA1 ↓ 10-fold
hbd ↓ 5.4-fold
crt2 ↓ 4.5-fold

Butyryl-CoA

ptb ↑ 83.4-fold
buk ↑ 170.1-fold

Butyrate

Stickland Metabolism

Proline | Glycine

Proline pathway
prdA ↑ 101.4-fold
prdB ↑ 112.5-fold
prdF ↑ 3986-fold

NAD

5-Aminovalerate

Glycine pathway
grdE ↑ 2.8-fold
grdC ↑ 2.7-fold
grdX ↓ 10-fold

→ NAD
→ ATP

Acetate

Electron bifurcation
bcd2 ↓ 11.7-fold
etfA2 ↓ 5-fold
etfB2 ↓ 3.7-fold

NAD FD_{red} | FD_{ox} NADH

NADH
ATP

Glucose utilization

Glucose → Pentose phosphate pathway

pfkA ↑ 45-fold
fbp ↑ 61.4-fold
fba ↑ 44.4-fold
tpi ↑ 38.5-fold
gapA ↑ 199.8-fold
gapB ↑ 22.4-fold
pgk ↑ 41.8-fold
eno ↓ 4.3-fold
pykF ↑ 55.9-fold
gpmI ↑ 15.2-fold

fbp ↑ 61.4-fold
tkt ↑ 12.3-fold
tkt' ↑ 67.3-fold
tal1 ↓ 2.3-fold
fba ↑ 44.4-fold
tpi ↑ 38.5-fold

Glycolysis / Gluconeogenesis

Pyruvate → Acetyl-CoA (Fermentation pathway)

ATP

C. difficile Fermentation and Energy Metabolism

Fermentation pathway

NAD → ATP

Rnf complex
rnfA ↑ 121.9-fold
rnfB ↑ 6-fold
rnfC ↑ 11.9-fold
rnfD ↑ 41.8-fold
rnfE ↑ 37.5-fold

H^+/Na^+

ATP Synthase
atpB ↓ 4.6-fold
atpE ↓ 8.5-fold
atpF ↓ 2.9-fold
atpI ↓ 7.1-fold
atpZ ↓ 7.2-fold

H^+/Na^+

Changes in gene expression in the presence of CH

↓ Gene down-regulated
↑ Gene up-regulated

# *C. hiranonis* massively altered *C. difficile* metabolism and toxin production

Due to their **high metabolic niche overlap**, *C. hiranonis* block *C. difficile*'s access to alternative resource niches and force them to undergo **massive metabolic alterations**, which also impact toxin production.

Adding other species in the community could enhance the inhibitory activity of *C. hiranonis*
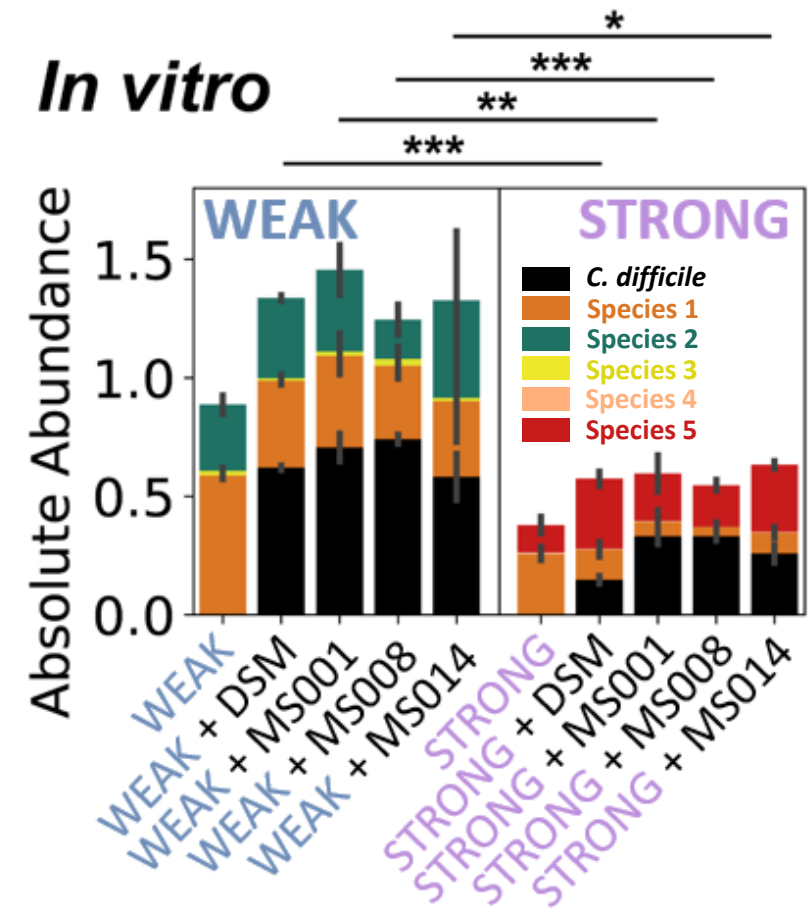
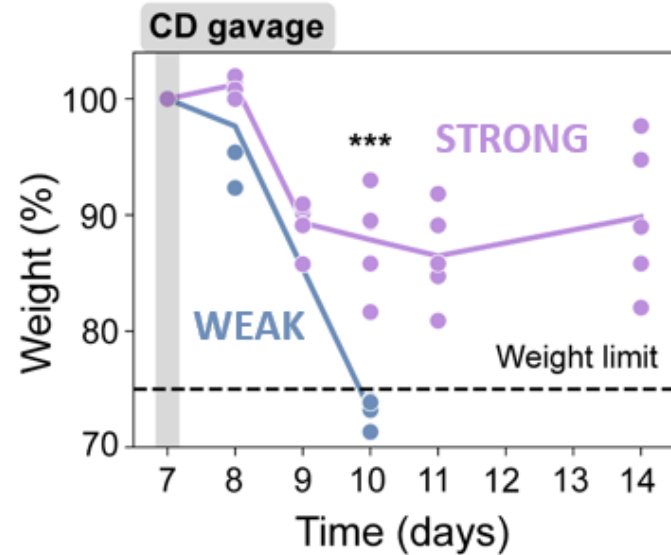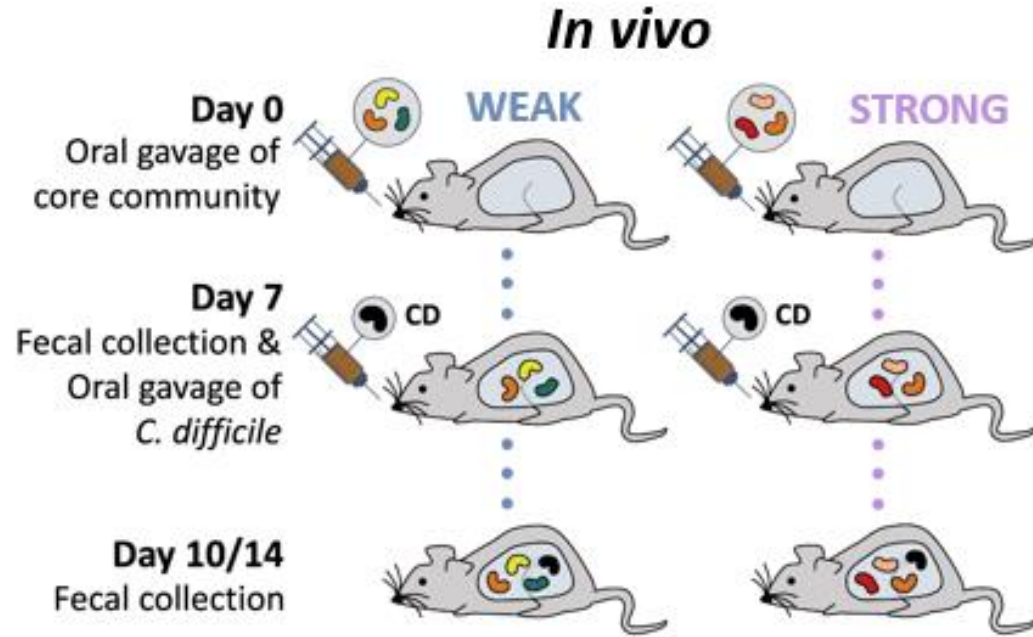# Predicting Strong and Weak *C. difficile*-inhibitory communities
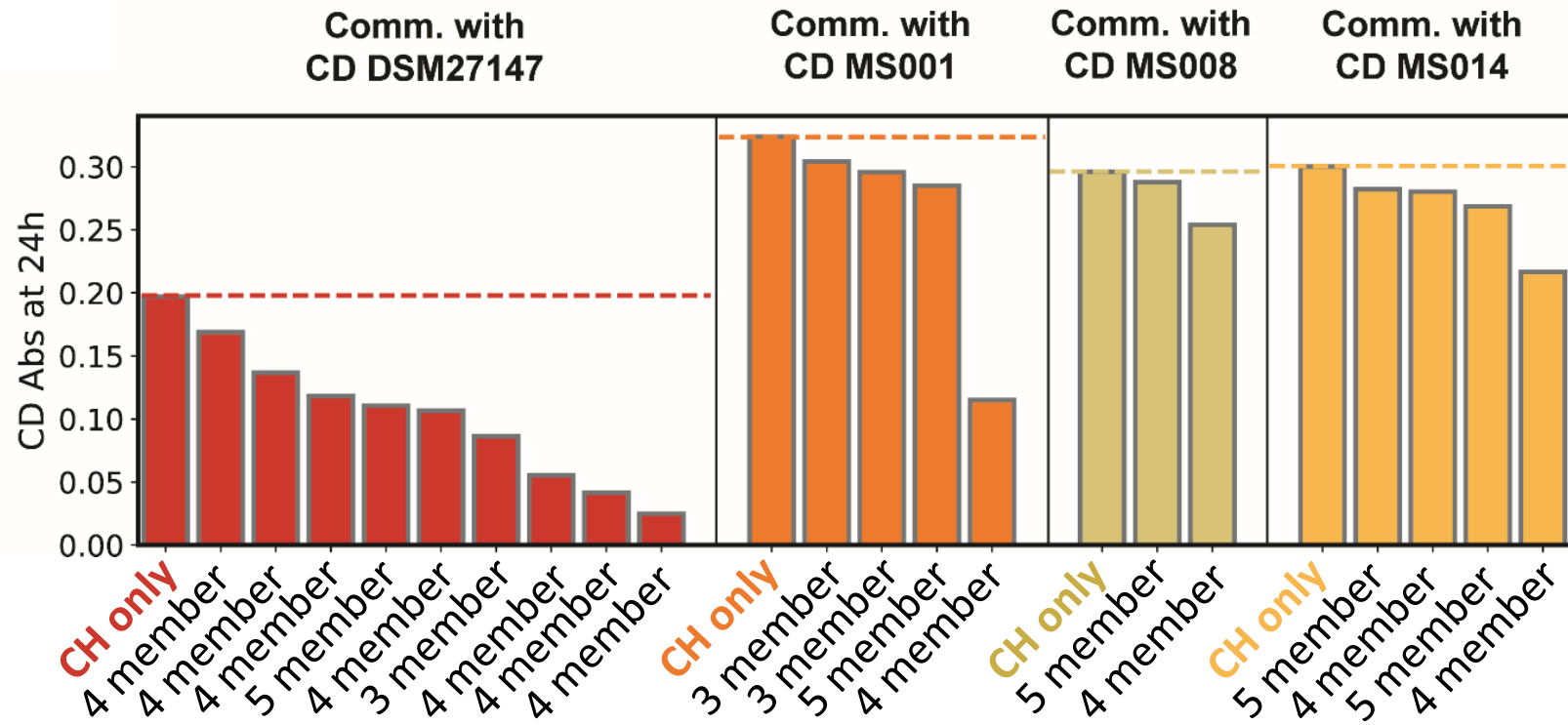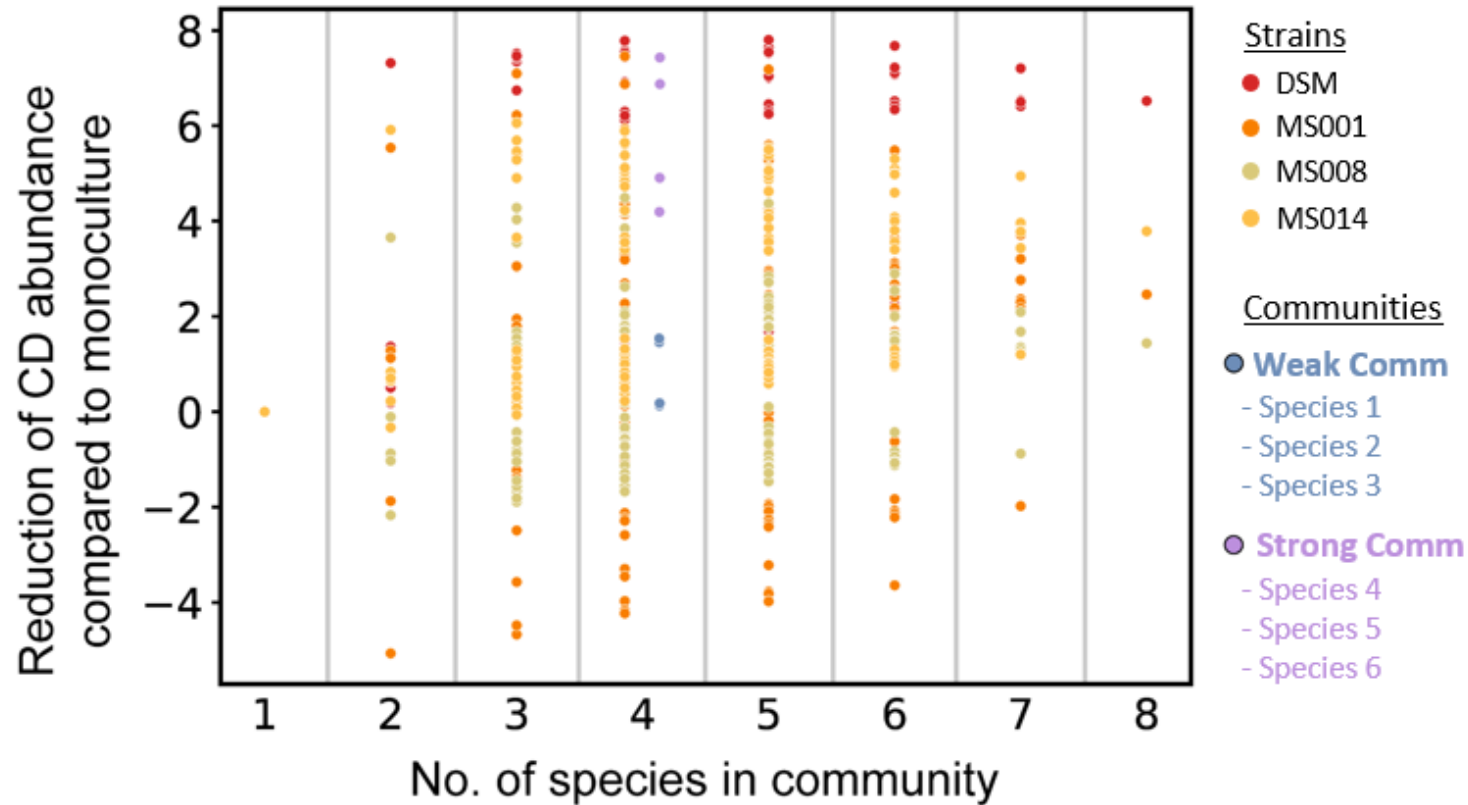
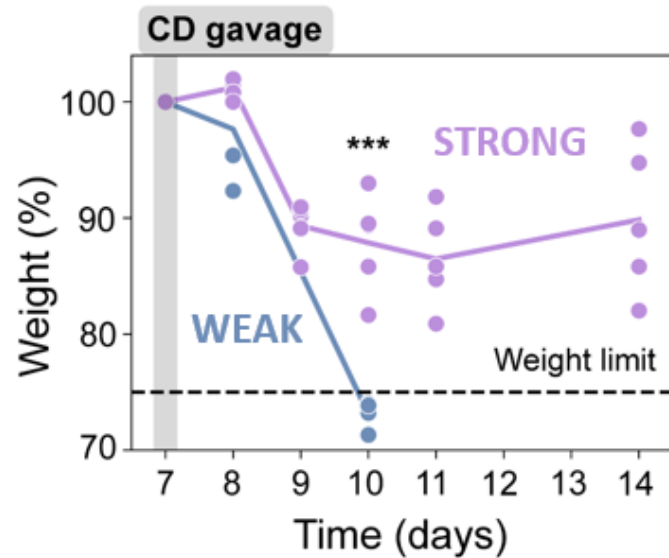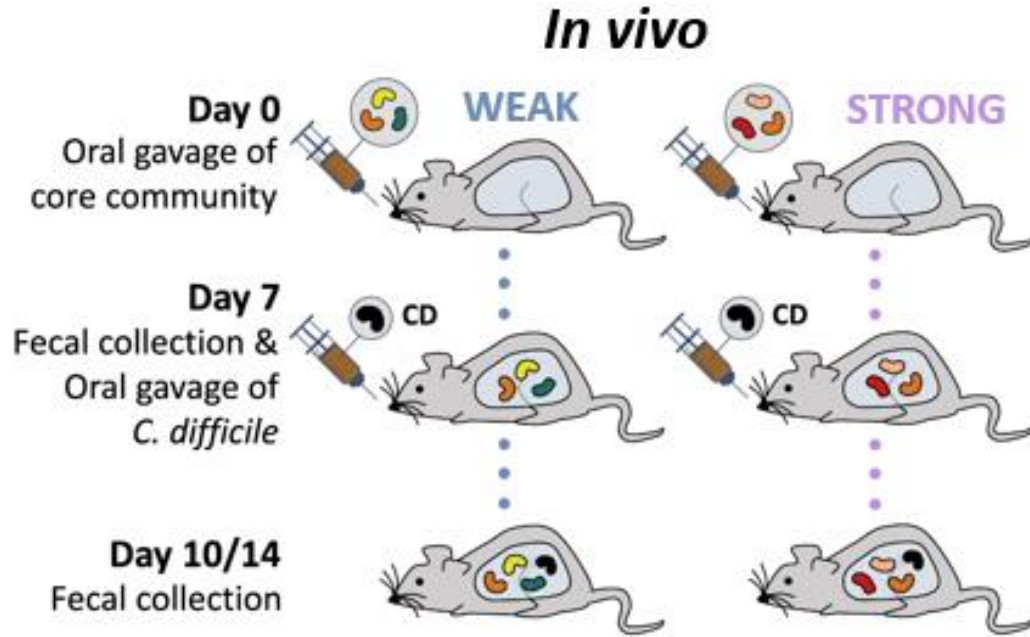# Predicting Strong and Weak *C. difficile*-inhibitory communities

Community inhibitory effects are consistent with computational model in germ-free mice

Community inhibitory effects are consistent with computational model in germ-free mice

55% *C. difficile*
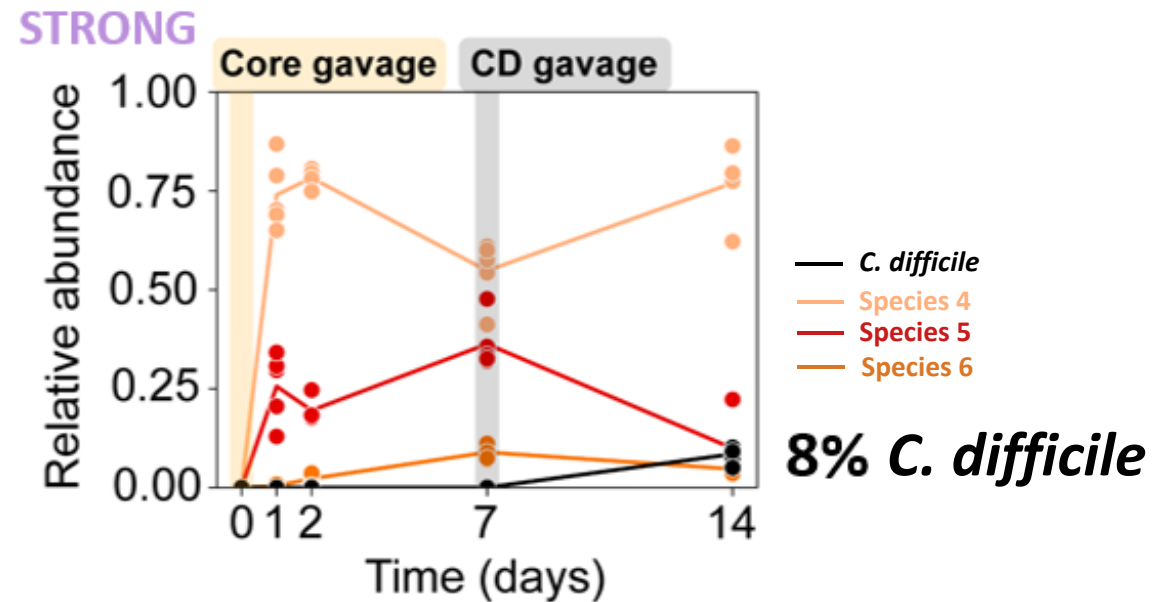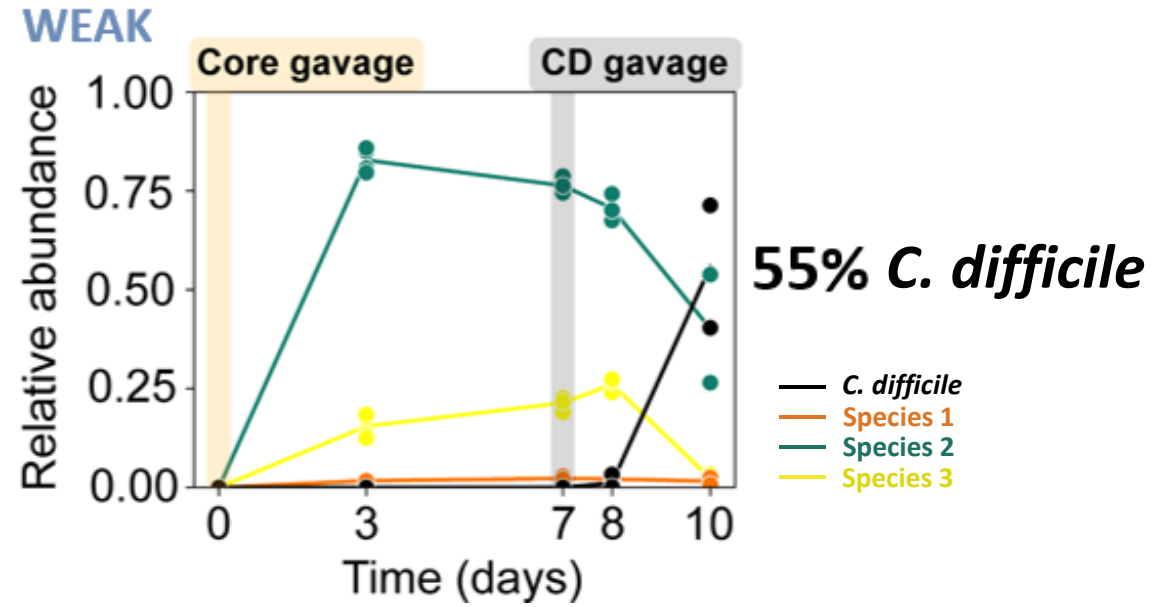
8% *C. difficile*

Conclusion

- Human gut bacteria infrequently inhibit *C. difficile* in the presence of preferred carbohydrates.

- *C. difficile* toxin production in communities is not explained by growth-mediated inter-species interactions.

- *C. hiranonis* is a "universal" *C. difficile* growth and toxin inhibitor that is robust against strain variation and nutrient environment.

- Model predicted 3-member community containing *C. hiranonis* protects mice from *C. difficile.*

Acknowledgment