Supplementary Material for Manuscript "Genome-level Constraint-Based Analysis of *Megasphaera sp. MJR8396C* Reveals Dual Metabolic Roles in the Human Gut"

1 Respiratory

The data sets and the code used to produce the material in the manuscript can be found on here.

2 Medium for pFBA Simulations

Table 1: Selected medium for pFBA simulations for evaluating nutrient composition's role in growth and metabolite synthesis. The table denotes constraints for metabolites with exchange reactions, expressed in mmol/gDW/h.

	Gut Medium	**AA-rich medium**	**Carbohydrates-rich medium**
L-Glutamate	9	10	9
Mg	7	7	7
Phosphate	6	6	6
Cl-	5	5	5
L-Proline	5	10	5
Urate	4	4	4
Octadecenoate	4	4	4
Palmitate	4	4	4
L-Leucine	4	10	4
Na+	4	4	4
L-Aspartate	4	10	4
Sucrose	3	3	10
Glycine	3	10	3
L-Serine	3	10	3
L-Alanine	3	10	3
L-Valine	3	10	3
L-Lysine	3	10	3
L-Phenylalanine	2	10	2
L-Isoleucine	2	10	2
L-Threonine	2	10	2
L-Arginine	2	10	2
D-Glucose	2	2	10
CO2	100	100	100
H2O	100	100	100
CELB	100	100	100
H2	100	100	100
K+	100	100	100
Ca2+	100	100	100
H+	100	100	100
Deoxycholate	1	1	1
ocdca	1	1	1

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Table 1 – continued from previous page

	Gut Medium	**AA-rich medium**	**Carbohydrates-rich medium**
Myristic acid	1	1	1
Butyrate	1	1	1
Propionate	1	1	1
D-Fructose	1	1	10
Acetate	1	1	1
L-Tyrosine	1	10	1
L-Methionine	1	10	1
L-Histidine	1	10	1
Tetrahydrofolate	0.1	0.1	0.1
Ethanol	0.1	0.1	0.1
Pyridoxal	0.1	0.1	0.1
Xylitol	0.1	0.1	0.1
Thiamin	0.1	0.1	0.1
octanoate	0.1	0.1	0.1
Decanoate	0.1	0.1	0.1
L-Tryptophan	0.1	10	0.1
Sorbitol	0.1	0.1	10
D-Mannitol	0.1	0.1	10
LACT	0.1	0.1	0.1
Galactose	0.1	0.1	10
Fe2+	0.1	0.1	0.1
Cu2+	0.1	0.1	0.1
PAN	0.1	0.1	0.1
L-Cysteine	0.1	10	0.1
BIOT	0.1	0.1	0.1
Fe3	0.1	0.1	0.1
Maltose	0.1	0.1	10
Co2+	0.1	0.1	0.1
Sulfate	0.1	0.1	0.1
Zn2+	0.1	0.1	0.1
Mn2+	0.1	0.1	0.1
Xylitol	0.1	0.1	10
Xylose	0	0	10
D-Mannose	0	0	10
Melibiose	0	0	10
L-Arabinose	0	0	10
D-Arabinose	0	0	10
Galactose	0	0	10
D-Mannose	0	0	10
D-Glucarate	0	0	10
Glycolate	0	0	10
Galacturonate	0	0	10

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	Gut Medium	**AA-rich medium**	**Carbohydrates-rich medium**
Galactonate	0	0	10

3 OptForce suggested interventions

Table 2: Must Decrease (mustL) Reactions

Reaction	**Name**	**Equation**
EX_cpd00036_e0	Succinate exchange	Succinate ↔
EX_cpd00309_e0	XAN exchange	XAN ↔
Bio1	Biomass	
rxn01637_c0	N2-Acetyl-L-ornithine:2-oxoglutarate aminotransferase	2-Oxoglutarate + N-Acetylornithine ↔ L-Glutamate + 2-Acetamido-5-oxopentanoate
rxn02465_c0	N-acetyl-L-glutamate-5-semialdehyde:NADP+ 5-oxidoreductase (phosphorylating)	NADP + Phosphate + 2-Acetamido-5-oxopentanoate ↔ NADPH + H+ + n-acetylglutamyl-phosphate
rxn05319_c0	TRANS-RXNBWI-115401.ce.maizeexp.OH_OH	H2O ↔ H2O
rxn05636_c0	TRANS-RXN-207.ce	H+ + Urate ↔ H+ + Urate
rxn05683_c0	Butyrate transport via proton symport, reversible	H+ + Butyrate ↔ H+ + Butyrate

Table 4: Must Increase (mustU) Reactions

Reaction	**Name**	**Equation**
EX_cpd00001_e0	H2O exchange	H2O ¡-¿
EX_cpd00009_e0	Phosphate exchange	P ¡-¿
EX_cpd00027_e0	Glucose exchange	Glucose ¡-¿
EX_cpd00300_e0	Urate exchange	C5H4N4O3 ¡-¿
Rxn00178_c0	Acetyl-CoA:acetyl-CoA C-acetyltransferase	2 Acetyl-CoA ¡-¿ CoA + Acetoacetyl-CoA
Rxn00192_c0	acetyl-CoA:L-glutamate N-acetyltransferase	Acetyl-CoA + L-Glutamate -¿ CoA + H+ + N-Acetyl-L-glutamate
Rxn00337_c0	ATP:L-aspartate 4-phosphotransferase	ATP + L-Aspartate ¡-¿ ADP + 4-Phospho-L-aspartate
Rxn00459_c0	2-phospho-D-glycerate hydro-lyase	2-Phospho-D-glycerate -¿ H2O + Phosphoenolpyruvate
Rxn00541_c0	L-threonine acetaldehyde-lyase	L-Threonine -¿ Glycine + Acetaldehyde
Rxn00692_c0	5,10-Methylenetetrahydrofolate:glycine hydroxymethyltransferase	H2O + Glycine + 5-10-Methylenetetrahydrofolate ¡-¿ L-Serine + Tetrahydrofolate
Rxn00802_c0	2-(Nomega-L-arginino)succinate arginine-lyase	L-Argininosuccinate -¿ L-Arginine + Fumarate
Rxn01019_c0	Carbamoyl-phosphate:L-ornithine carbamoyltransferase	Ornithine + Carbamoylphosphate ¡-¿ Phosphate + H+ + Citrulline
Rxn01069_c0	O-phospho-L-homoserine phosphate-lyase	H2O + O-Phospho-L-homoserine -¿ Phosphate + L-Threonine
Rxn01300_c0	ATP:L-homoserine O-phosphotransferase	ATP + L-Homoserine -¿ ADP + H+ + O-Phospho-L-homoserine
Rxn01434_c0	L-Citrulline:L-aspartate ligase	ATP + L-Aspartate + Citrulline -¿ PPi + AMP + 2 H+ + L-Argininosuccinate
Rxn01522_c0	xanthine:NAD+ oxidoreductase	H2O + NAD + XAN ¡-¿ NADH + H+ + Urate
Rxn01917_c0	ATP:N-acetyl-L-glutamate 5-phosphotransferase	ATP + N-Acetyl-L-glutamate ¡-¿ ADP + n-acetylglutamyl-phosphate
Rxn05202_c0	TRANS-RXN-206.ce	H+ + XAN ¡-¿ H+ + XAN
Rxn31312	'2-oxoglutarate synthase'	CoA + 2-Oxoglutarate + Oxidizedferredoxin → CO2 + H+ + Succinyl-CoA + Reducedferredoxin

4 *In Silico* Simulation of Microbe-microbe Interactions

Table 6: Community constraints applied in FBA simulations to evaluate the cross-feeding potential of acetate and D-lactate within a community comprising *Bifidobacterium adolescentis* and *Megasphaera sp. MJR8396C* These constraints are derived from individual simulations utilizing minimal medium and are specified for metabolites with exchange reactions, quantified in mmol/gDW/h.

	Community constraints	***Bifidobacterium adolescentis* constraints**	***Megasphaera sp.* constraints**
Pyridoxal	0.003947	-	0.003947
Riboflavin	0.010331	0.010331	-
PAN	0.010331	0.010331	-
Nicotinamide	0.010331	0.010331	-
Thiamin	0.009112	0.005165	0.003947
Tetrahydrofolate	0.015496	0.015496	-
MN2+	0.014588	0.007294	0.007294
Zn2+	0.014588	0.007294	0.007294
Cu2+	0.014588	0.007294	0.007294
Ca2+	0.014588	0.007294	0.007294
Cl-	0.014588	0.007294	0.007294
Mg	0.014588	0.007294	0.007294
fe3	0.014588	0.007294	0.007294
K+	0.014588	0.007294	0.007294
Fe2+	0.022482	0.007294	0.015188
L-tryptophan	0.063239	0.063239	-
L-tryosine	0.128856	0.128856	-
L-Phenylalanine	0.204458	0.204458	-
L-Histidine	0.089931	-	0.089931
ocdca	0.16789	0.084654	0.083236
L-cysteine	0.2	0.1	0.1
L-Lysine	0.32575	-	0.32575
L-Leucine	0.510908	-	0.510908
Phosphate	3.261986	2.255919	1.006067
D-Glucose	2	-	2
L-Arginine	2	-	2
L-Methionine	2	1	1
Glycine	3	-	3
L-Alanine	3	-	3
L-Serine	3	-	3
Sucrose	3	-	3
L-aspartate	4	-	4
L-Threonine	4	2	2
Urate	4	-	4

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	Community constraints	***Bifidobacterium adolescentis* constraints**	***Megasphaera sp.* constraints**
L-Glutamate	9	9	9
CELB	100	100	-
Co2+	0	0.007294	0.007294
Acetate	-	-	100
D-Lactate	-	-	100

5 Pairwise interactions

Organisms can exert positive, negative, and neutral effects on each other. Negatively, competition (-/-) diminishes the functioning of both species involved, whereas amensalism (-/0) harms one species while the other remains unaffected. Conversely, on the positive side, parasitism (+/-) benefits one member at the expense of the other, commensalism (+/0) benefits one member while having no effect on the other, and mutualism (+/+) benefits both species involved. By using a similar approach to *Heinken et al.*, pairwise interactions were simulated for *Megasphaera sp. MJR8396C* and *Bifidobacterium adolescentis.* The pairwise interactions were assessed across various media to determine their susceptibility to nutrient availability. The simulated media included a gut medium, an amino acid-rich medium, a carbohydrate-rich medium, as well as adaptations of a Western diet and a high-fiber diet, sourced from *Magnusdottir et al.*. The results are given in Table 7. Across all media except the amino acid-rich medium, the interaction exhibited amensalism, characterized by *Bifidobacterium's* growth remaining hardly affected while the growth rate of *Megasphaera sp. MJR8396C* dropped to almost negligible levels. However, for the interaction involving the amino acid-rich medium, a parasitic relationship emerged. Here, the growth of *Megasphaera sp. MJR8396C* increased significantly while *Bifidobacterium's* growth decreased, suggesting that *Megasphaera sp. MJR8396C* prospers at the expense of *Bifidobacterium.*

Table 7: Growth rates of *Bifidobacterium adolescentis* and *Megasphaera sp. MJR8396C* in different media and their interactions.

Medium	**Paired Growth B.**	**Paired Growth M.**	**Single Growth B.**	**Single Growth M.**	**Interaction**
Gut Medium	1.402	0.001	1.403	1.114	Amensalism
Amino Acid-Rich Medium	0.075	5.864	4.472	2.056	Parasitism
Carbohydrate Rich Medium	1.402	0.001	1.403	1.114	Amensalism
Western Diet	1.402	0.001	1.403	1.114	Amensalism
High Fiber Diet	1.402	0.001	1.403	1.114	Amensalism

Table 9: Additional Western Diet (WD) and High Fiber diet (HF) medium for pairwise interaction simulations, adapted from Magnúsdóttir et al. and adjusted to gut medium. The table denotes constraints for metabolites with exchange reactions, expressed in mmol/gDW/h.

Metabolite	**WD adjusted to GM**	**HF adjusted to GM**
H2O	100	100
Phosphate	6	6
CO2	100	100
L-Glutamate	9	9
D-Glucose	10	2
Acetate	1	1
MN2+	0.1	0.1
Glycine	3	3
Zn2+	0.1	0.1
L-Alanine	3	3
L-Lysine	3	3
L-aspartate	4	4
SO4	0.1	0.1
L-Arginine	2	2
L-Serine	3	3
Cu2+	0.1	0.1
L-Methionine	1	1
Ca2+	100	100
L-tryptophan	0.1	0.1
L-Phenylalanine	2	2
H+	100	100
L-tryosine	1	1
Sucrose	10	3
D-fructose	10	1
L-cysteine	0.1	0.1
Tetrahydrofolate	0.1	0.1
Cl-	5	5
Glycerol	10	0
BIOT	0.1	0.1
D-ribose	10	0.1
L-Leucine	4	4
Galactose	10	0.1
L-Histidine	1	1
L-Proline	5	5
Nicotinamide	0.1	0.1
D-Mannose	10	0.1
Propionate	1	1
Co	0.1	0.1
Xylose	10	0.1
L-valine	3	3

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Metabolite	**WD adjusted to GM**	**HF adjusted to GM**
CELB	100	100
L-Threonine	2	2
Maltose	10	0.1
K+	100	100
LACT	0.1	0.1
Butyrate	1	1
Palmitate	4	4
Pyridoxal	0.1	0.1
Riboflavin	0.1	0.1
L-Arabinose	10	0.1
Mg	7	7
Urate	4	4
Thiamin	0.1	0.1
Xylitol	0.1	0.1
D-Mannitol	10	0.1
L-Isoleucine	2	2
Ethanol	0.1	0.1
L-Rhamnose	10	0.1
Sorbitol	0.1	0.1
PAN	0.1	0.1
L-Fucose	10	0.1
Na+	4	4
ocdca	1	1
Decanoate	0.1	0.1
Glycocholate	1	1
beta-lactose	10	0.1
Chemodeoxycholate	1	1
Deoxycholate	1	1
Taurocholate	1	1
Melibiose	10	0.1
Taurochenodeoxycholate	1	1
Glycochenodeoxycholate	1	1
octanoate	10	0.1
Myristic Acid	1	1
Fe2+	0.1	0.1
Fe3	0.1	0.1
H2	100	100
hexadecenoate	10	0.1
octadecenoate	10	4
tetradecenoate	10	0.1
starch (n=27)	10	0.1
starch (n=19)	0.1	10

6 BacArena

In addition, BacArena simulations were utilized to deepen the understanding of interaction mechanisms between *Megasphaera sp. MJR8396C* and *Bifidobacterium adolescentis* within a standard gut medium and an amino-acid-enriched medium. The simulations spanned a 24-hour period, providing insight into the growth dynamics of the microbial communities. Figures 1 and 2 present the resulting growth curves and the associated variations in metabolite concentrations throughout the simulation.

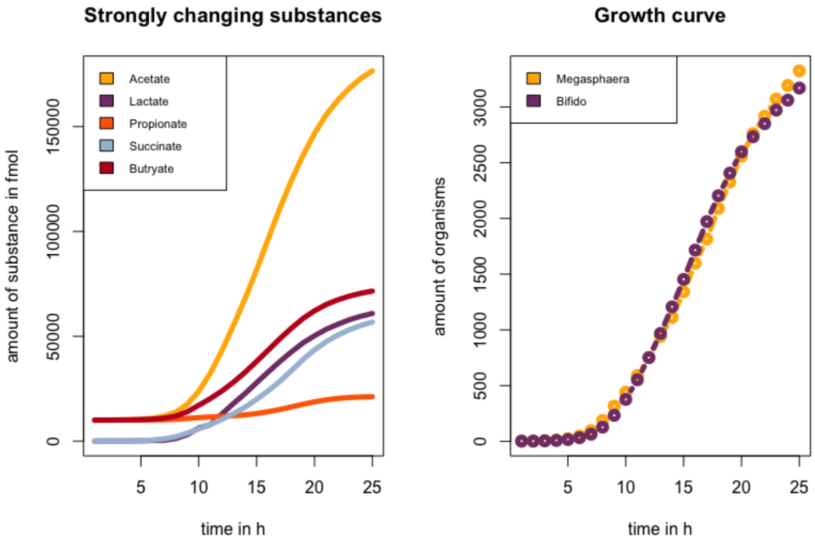

Figure 1: BacArena simulations conducted for *Megasphaera* MJR8396C and *Bifidobacterium adolescentis* across a 24-hour period using gut medium.

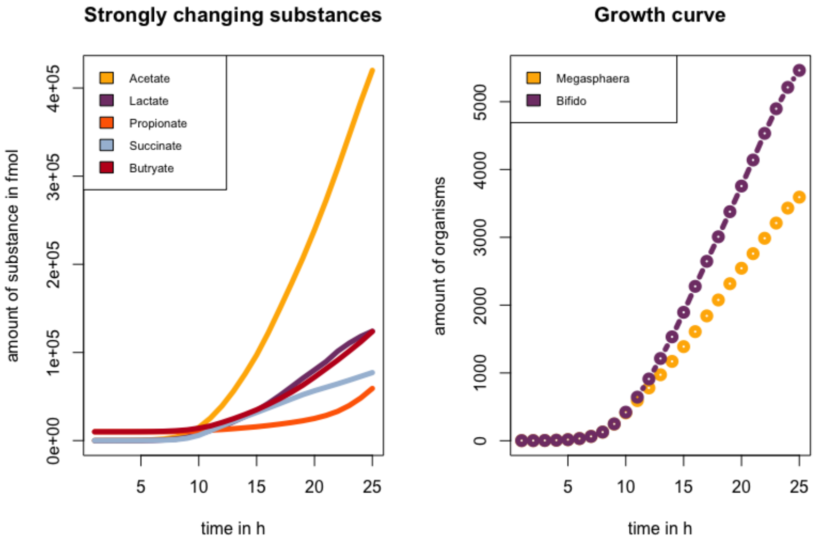

Figure 2: BacArena simulations conducted for *Megasphaera* MJR8396C and *Bifidobacterium adolescentis* across a 24-hour period using amino acid-rich medium.

Both *Megasphaera sp. MJR8396C* and *Bifidobacterium adolescentis* demonstrated growth, as evidenced by the growth curves showing logarithmic or exponential phases typical in nutrient-rich environments. In the gut medium, both organisms exhibited similar growth patterns; however, in the amino acid-rich medium, *Bifidobacterium*'s abundance slightly increased. The temporal profiles of lactate and butyrate production were observed to mirror each other, suggesting a synchronized pattern across the different media. Particularly in an amino acid-enriched medium, these production pathways seemed more closely aligned, with notable increases in lactate production coinciding with a higher presence of *Bifidobacterium* organisms. Acetate production was consistently high in both media types. Additionally, the presence of amino acids was associated with increased levels of lactate, propionate, succinate, and butyrate, reflecting enhanced microbial activity.