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Supplemental information

Modeling *Drosophila* gut microbe interactions reveals metabolic interconnectivity

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Supplemental figures and legends

A

	Exp. growth rate [1/h]			In silico growth rate [1/h]		
	MRS	ACE	HD	MRS	ACE	HD
L. plantarum A2	0.26	0.11	0.13	0.24	0.26	0.36
L. plantarum B2	0.25	0.09	0.07	0.26	0.24	0.39
L. brevis B6	0.11	0.09	0.14	0.11	0.11	0.12
A. indonesiensis A4	0.10	0.07	0.20	0.29	0.30	0.08
A. indonesiensis A5	0.10	0.10	0.18	0.30	0.30	0.08
A. pasteurianus B5	0.05	0.05	0.20	0.28	0.30	0.09

B

$$\mu_{exp} = \frac{\ln(OD600_t) - \ln(OD600_{t_0})}{t - t_0}$$

$$\mu_{in\ silico} = \frac{\ln\left(\frac{biomass_t}{biomass_{t_0}}\right)}{t - t_0}$$

C

	MRS	ACE	HD
L. plantarum A2	0.02	-0.15	-0.24
L. plantarum B2	-0.01	-0.15	-0.31
L. brevis B6	0.00	-0.01	0.02
A. indonesiensis A4	-0.19	-0.23	0.12
A. indonesiensis A5	-0.20	-0.20	0.10
A. pasteurianus B5	-0.23	-0.25	0.11

In silico higher growth than experiment (delta ≥ - 0.2)

In silico elevated growth than experiment (delta > - 0.15 < - 0.2)

In silico and experimental growth comparable (delta ~ 0)

Experimental growth higher than *in silico* (delta ≥ 0.1)

Figure S1. Growth estimate determination and comparison between wet-lab and *in silico* results, Related to Fig. 1 and Fig. 2. (A) The growth rate of the six isolated bacteria was estimated on the three different media (MRS, ACE and HD) by wet-lab experiments (left part of the table) and using the computed biomass production amounts to calculate *in silico* derived growth rates (right side of the table). The formulas used are provided in (B). (C) We subtracted from the experimentally determined growth rate the *in silico* derived one to get a similarity measure (difference is “delta”). The color-coded delta values obtained are provided in the table.

		MRS	ACE	HD
SUBSTRATE	CPD	Conc. [mM]	Conc. [mM]	Conc. [mM]
GLUCOSE	cpd00027	111.00000	55.50000000	-
SUCROSE	cpd00076	-	-	50
GLUTAMINE	cpd00053	0.06842	0.10947657	12.47000000
GLUTAMATE	cpd00023	19.47937	13.36912934	12.40000000
PHENYLALANINE	cpd00066	4.64919	3.80168291	5.55000000
HISTIDINE	cpd00119	2.27507	1.44367105	4.46000000
LYSINE	cpd00039	7.98960	6.00588276	11.89000000
METHIONINE	cpd00060	1.82976	1.13270777	2.27000000
ARGININE	cpd00051	5.92423	6.18828932	8.17000000
THREONINE	cpd00161	4.58361	2.45970450	10.86000000
VALINE	cpd00156	8.02390	5.97524541	11.41000000
TRYPTOPHAN	cpd00065	0.74426	0.41619742	2.15000000
LEUCINE	cpd00107	9.41526	6.84607761	12.50000000
ISOLEUCINE	cpd00322	5.72539	4.23115042	8.84000000
ALANINE	cpd00035	17.15120	20.51857672	17.82000000
ASPARTIC ACID	cpd00041	10.70624	8.82043576	6.32000000
ASPARAGINE	cpd00132	0.68120	0.94610960	6.35000000
GLYCINE	cpd00033	26.22885	34.96736379	14.43000000
TYROSINE	cpd00069	1.58949	1.02654672	4.64000000
CYSTEINE	cpd00084	0.18727	0.06658344	2.18000000
SERINE	cpd00054	6.34694	3.35902560	7.23000000
PROLINE	cpd00129	14.51403	12.85503344	4.90000000
K+	cpd00205	34.33960	7.49150219	22.00000
CA2	cpd00063	0.05115	0.03717750	1.14000
ZN2	cpd00034	0.01000	0.01000000	0.08700
FE+2	cpd10515	0.004895	0.00501	0.04495
FE+3	cpd10516	0.004895	0.00501	0.04495
RIBOFLAVIN	cpd00220	0.01000	0.01000000	0.00186
PHOSPHATE	cpd00009	6.34404	3.38629687	22.00000
MAGNESIUM	cpd00254	1.90167	0.25735445	2.08000
MN2+	cpd00030	0.33128	0.01000000	0.00505
BIOTINE	cpd00104	0.01000	0.01000000	0.00060
CU2	cpd00058	0.01000	0.01000000	0.01000
CL-	cpd00099	3.28728	4.50289115	2.64830
PANTOTHENATE	cpd00644	0.01000	0.01000000	0.04949
THIAMIN	cpd00305	0.01000	0.01000000	0.00465
SULFATE	cpd00048	1.77389	0.57464085	2.16687
FOLIC ACID	cpd00393	0.01000	0.01000000	0.00113
URIDINE	cpd00249	0.01000	0.01000000	0.24600
INOSINE	cpd00246	0.01000	0.01000000	0.24200000
NICOTINIC ACID	cpd00218	0.01000	0.01000000	0.06820000
PYRIDOXINE	cpd30645	0.01000	0.01000000	0.01000000
NA+	cpd00971	4.68312	4.49582427	11.90500000
O2	cpd00007	0.10000	0.1	0.10000000
WATER	cpd00001	100.00000	100.00000000	100.00000000
MOLYBDATE	cpd11574	0.01000	0.01000000	-
CO2+	cpd00149	0.01000	0.01000000	0.01
AMMONIUM	cpd00013	8.22335	-	-
CITRATE	cpd00137	8.22335	-	-
ACETATE	cpd00029	59.91674	52.40000000	-
ETHANOL	cpd00363	-	85.60000000	-
CHOLINE	cpd00098	-	-	0.35810
MYO-INOSITOL	cpd00121	-	-	0.02800

Figure S2. Parametrization of the media contents used in this study, Related to Table 1, STAR Methods, and Supplemental File 2. The “CPD” ID refers to the compound descriptor used in the modeling simulations.

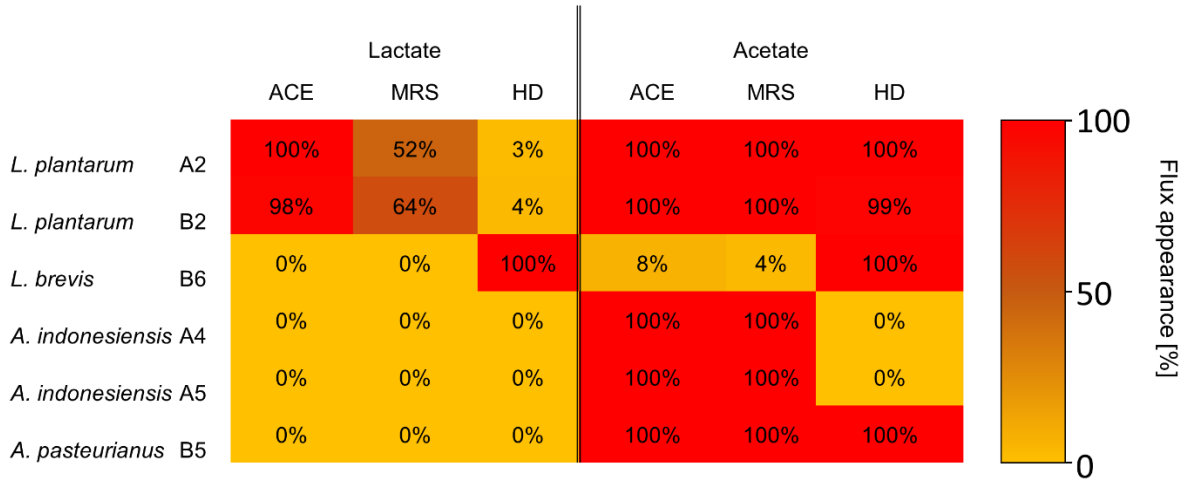

Figure S3. Robustness analysis of the signature metabolite production of *Lactobacilli* and *Acetobacter* bacteria on the three different media, Related to Fig. 3. Simulations were run 100 times and the production of lactate and acetate, respectively, was recorded. As a threshold, metabolite production had to surpass a flux rate of 0.1 nmol / h to count as “flux present”. The plots show the color-coded results with red color representing a high fraction of simulation runs with metabolite production and orange shades represent a low fraction of metabolite producing simulation runs.

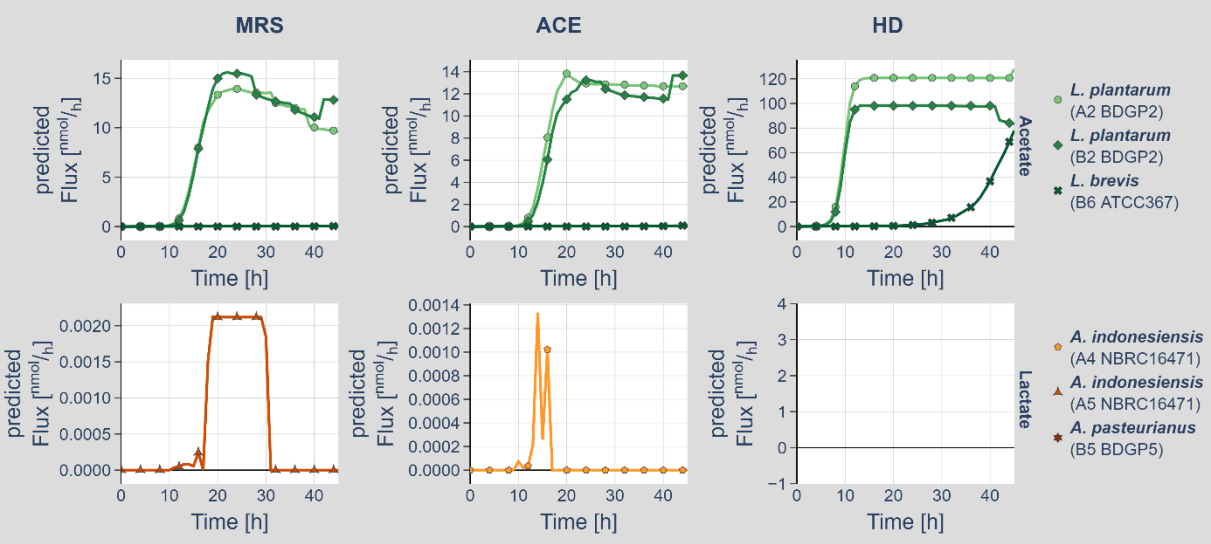

Figure S4. Test for heterolactic behavior of *Acetobacter* and *Lactobacillus* models, Related to Fig. 3. While all *Lactobacilli* were able to produce also acetate (upper row), the *Acetobacter* models failed to produce lactate exceeding our 0.1 nmol / h flux threshold.

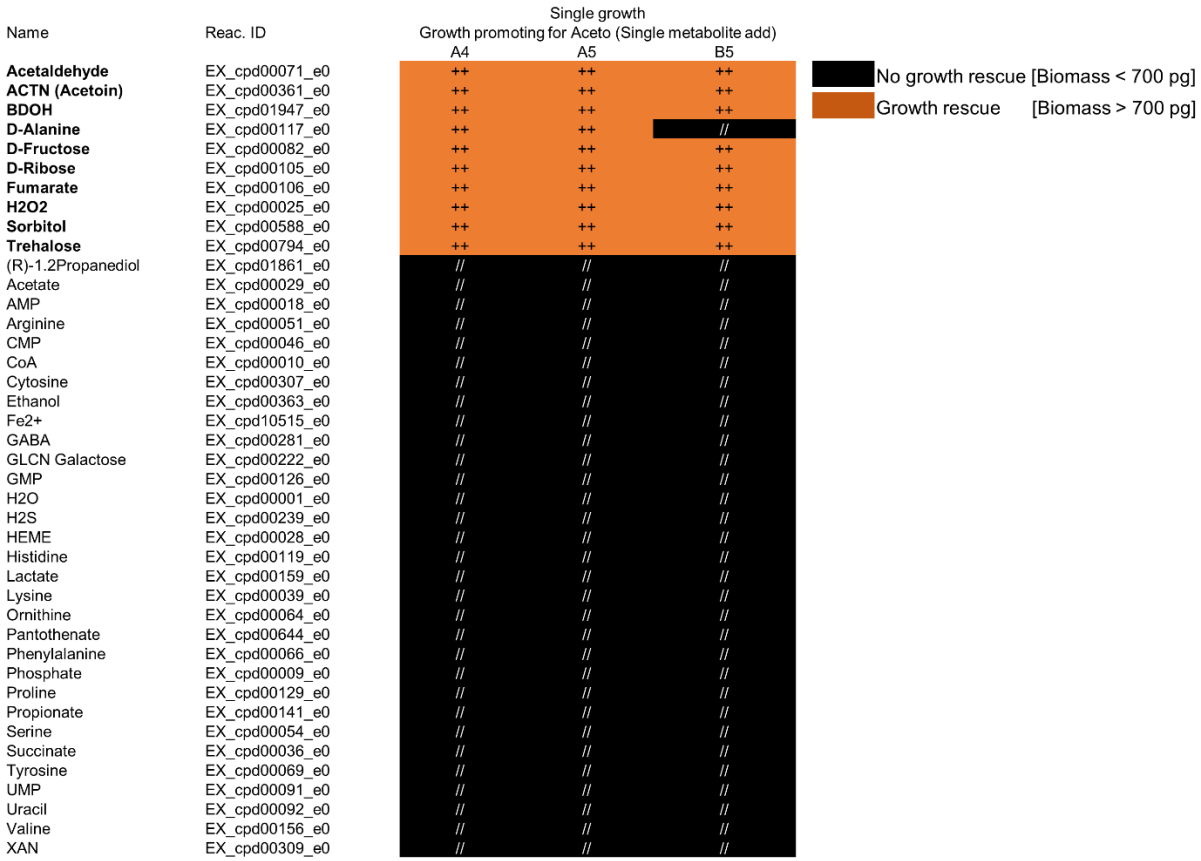

Figure S5. Rescue of the *Acetobacter sp.* growth deficit in the HD by adding singular metabolites, Related to Fig. 5 and Fig. 6. All metabolites which showed an exchange behavior in the combined growth simulations (cf. Figure 5A and supplemental file 2) were added individually to the HD simulations. The simulations were evaluated for a rescue of the growth deficit which is represented by red color in the table. The majority of added metabolites did not alter the biomass production of the model (represented by black color).

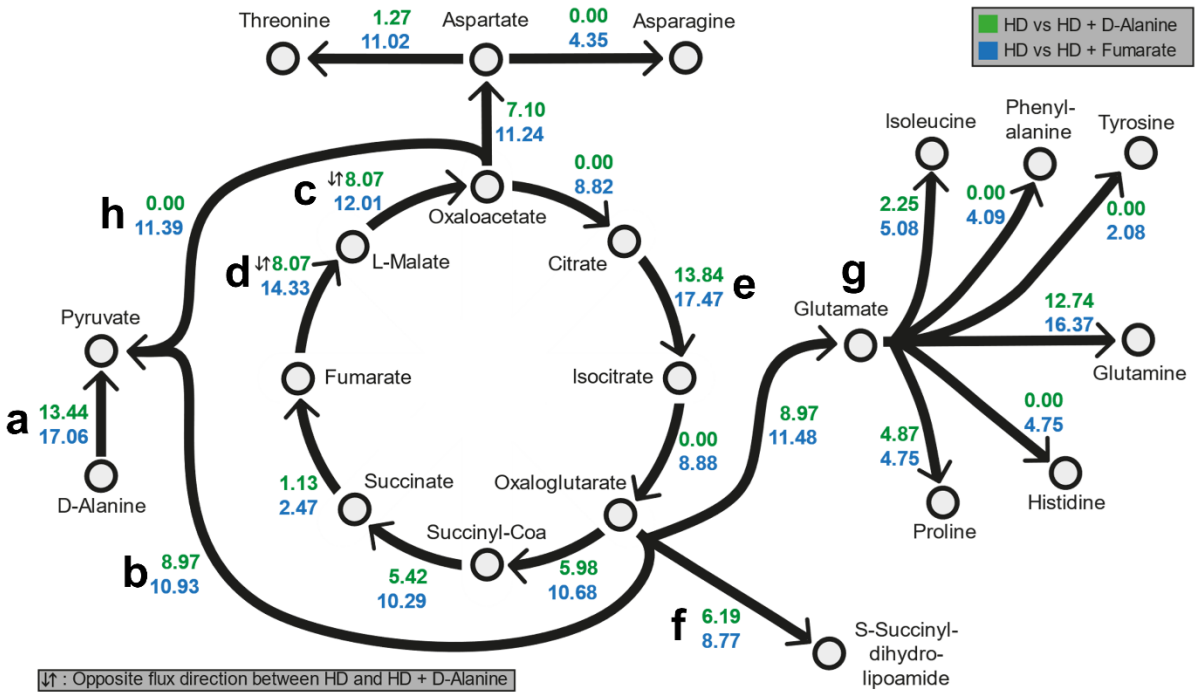

Figure S6. Focused metabolic network with flux differences of *A. pasteurianus* (B5) growth modeled on HD or HD with D-Alanine or HD with fumarate, respectively, Related to Fig. 6. Flux changes were calculated by subtracting the flux present on the HD from the value present in the HD with additive. The result was log normalized. Highlighted reactions are (if multiple reactions are provided, the individual flux values were combined to produce one summarizing value): a) L-Alanine racemase and L-alanine:glyoxylate aminotransferase, b) L-Alanine:2-oxoglutarate aminotransferase, c) Malate-dehydrogenase, d) Fumarase, e) Aconitase, f) 2-oxoglutarate dehydrogenase g) glutamate to isoleucine: L-Isoleucine:2-oxoglutarate aminotransferase; glutamate to phenylalanine: L-Phenylalanine:2-oxoglutarate aminotransferase; glutamate to tyrosine: L-tyrosine:2-oxoglutarate aminotransferase; glutamate to glutamine: L-Glutamate:ammonia ligase (ADP-forming) and L-glutamine:D-fructose-6-phosphate isomerase (deaminating); glutamate to histidine: L-Histidinol-phosphate phosphohydrolase and L-Histidinol:NAD+ oxidoreductase; glutamate to proline: ATP:L-glutamate 5-phosphotransferase and L-glutamate-5-

semialdehyde:NADP+ 5-oxidoreductase (phosphorylating) and L-glutamate 5-semialdehyde dehydratase and L-Proline:NADP+ 5-oxidoreductase, h) oxaloacetate carboxy-lyase (pyruvate-forming).