

Figure S1. Heatmap describing supernatant metabolomes for all mono- and co-cultures after growth (related to Figure 3). Red indicates higher concentration than fresh medium, while blue indicates lower concentration. Values are centered at 0 using the mean value in fresh media, then scaled between -1 and +1 by dividing by the maximum change in concentration for each metabolite in any sample in the study. Only metabolites for which an identity could be determined are shown. Hierarchical clustering of metabolites was performed using Euclidean distances and complete linkage.

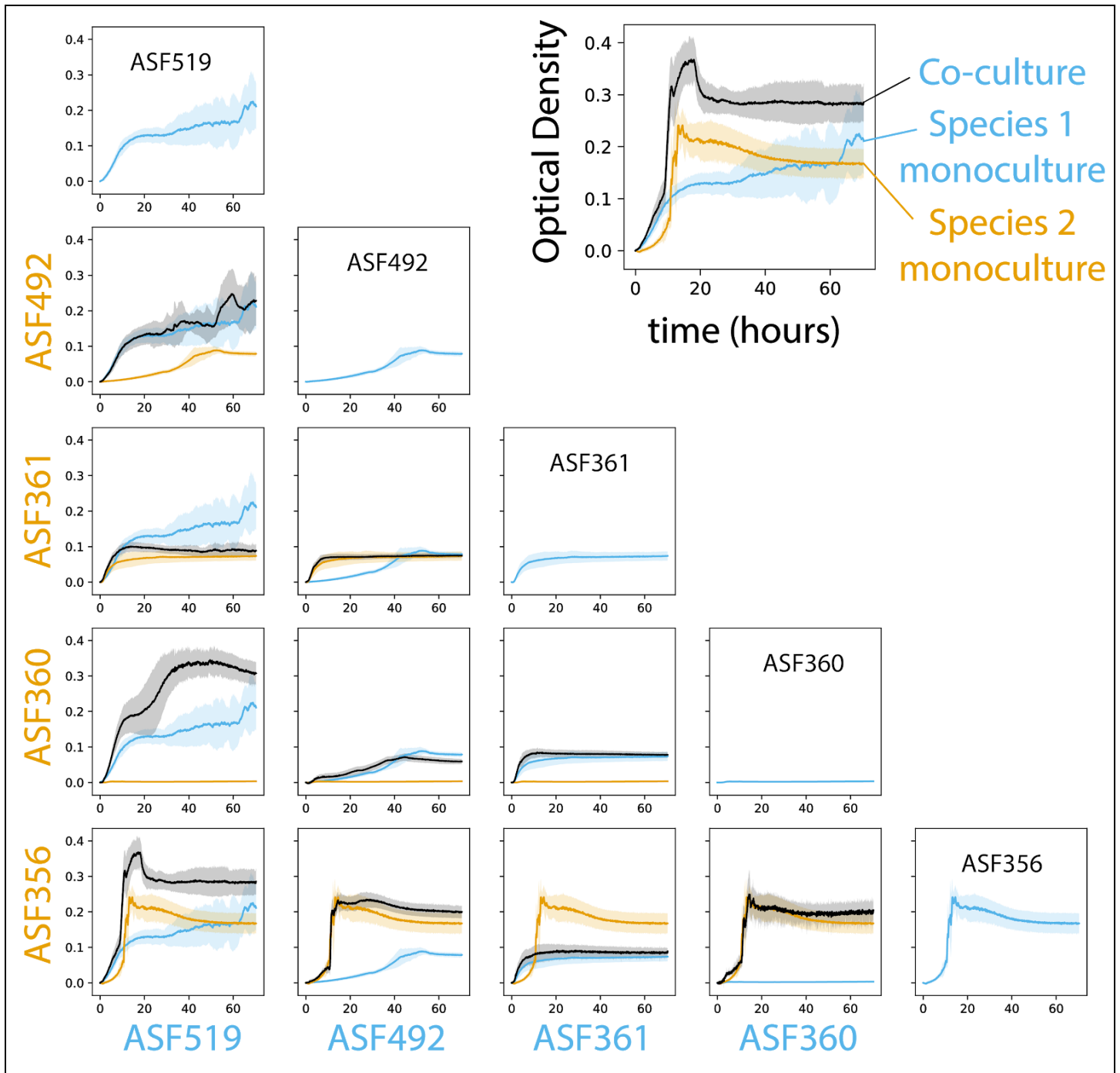
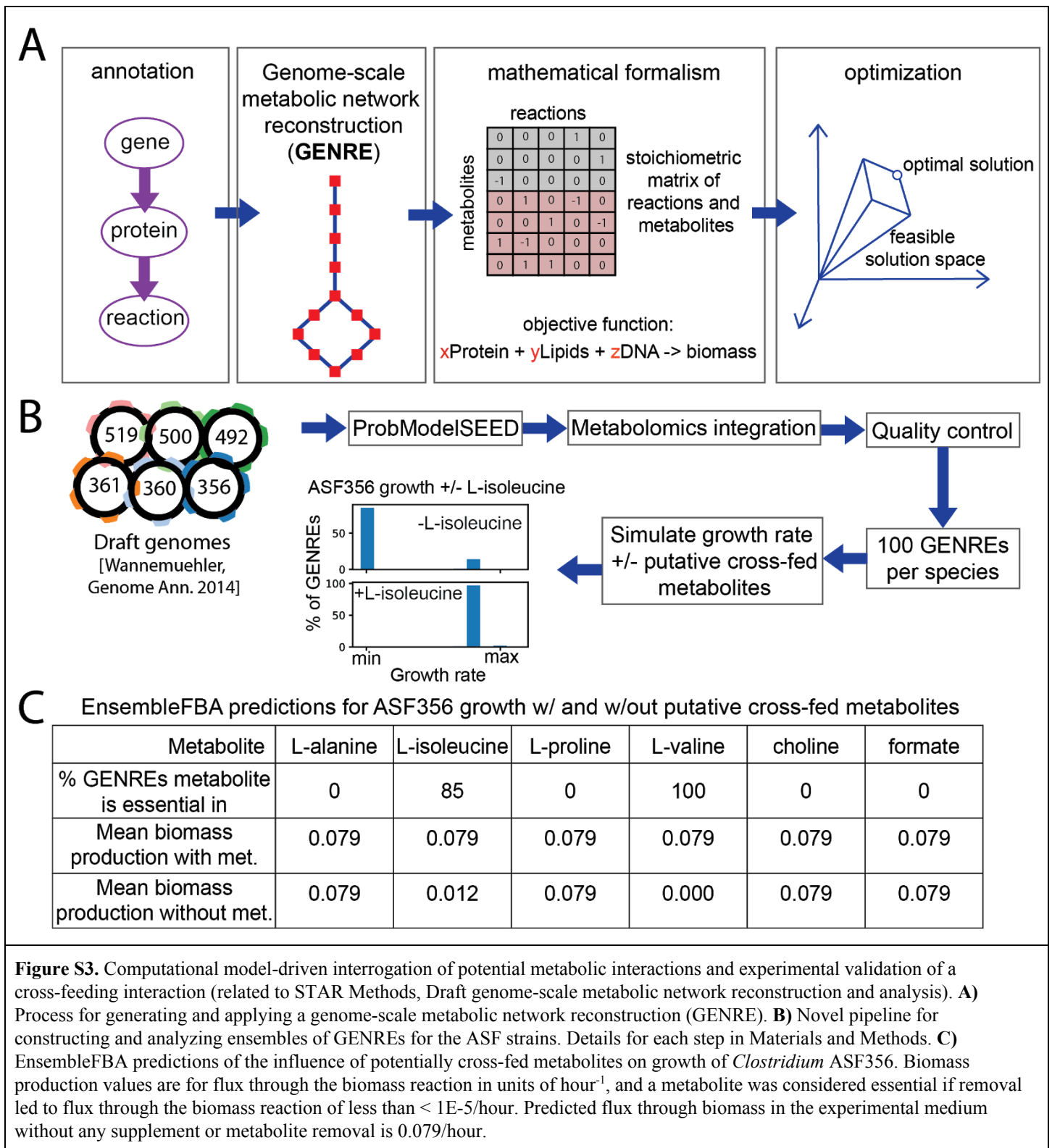


Figure S2. Optical density-based growth curves for *Clostridium* ASF356, *Lactobacillus* ASF360, *Lactobacillus* ASF361, *Eubacterium* ASF492, and *Parabacteroides* ASF519 (related to Figures 1 and 2). Optical density was measured at 589nm. Experiments were performed in 96 well plates with 200uL total volume in each well. Each sample group (monocultures and co-cultures) contains 8 biological replicates from a single experiment (e.g. each replicate was grown in an independent well, but they were derived from the same starter culture). Line shows the mean for each sample group, and shading extends one standard deviation from the mean in both the positive and negative direction. Sky blue line indicates monoculture for the strain labelled in sky blue along the x axis. Orange line indicates monoculture for the strain labelled in orange along the y axis. Black line indicates co-culture of the two strains. Diagonal shows the monoculture growth curve for each species. Axes units are identical on all subplots. Time is shown in hours, extending to 72 hours.



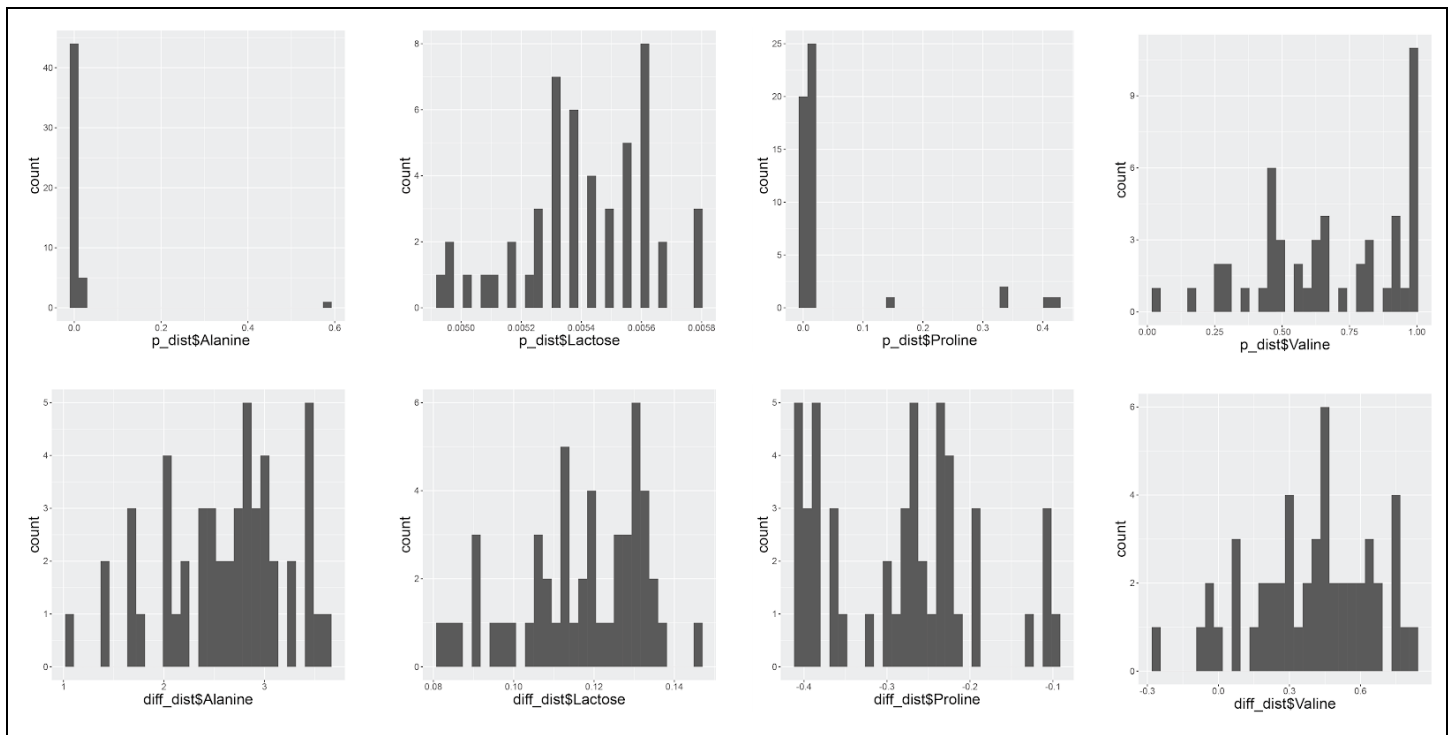


Figure S4. Selected examples of ConYE results using bootstrapped strain abundances (related to STAR Methods, Constant Yield Expectation (ConYE) model). Top row shows the distribution of ConYE p -values for each metabolite and bottom row shows the distribution of difference from the expected value for the same metabolite within each column. Examples are from the co-culture of *Clostridium* ASF356 and *Parabacteroides* ASF519. Distributions were generated by recalculating the average abundance of each strain in monoculture using leave-two-out bootstrapped samples prior to calculating the monoculture yield for each metabolite. N=50 samples with replacement for each monoculture.

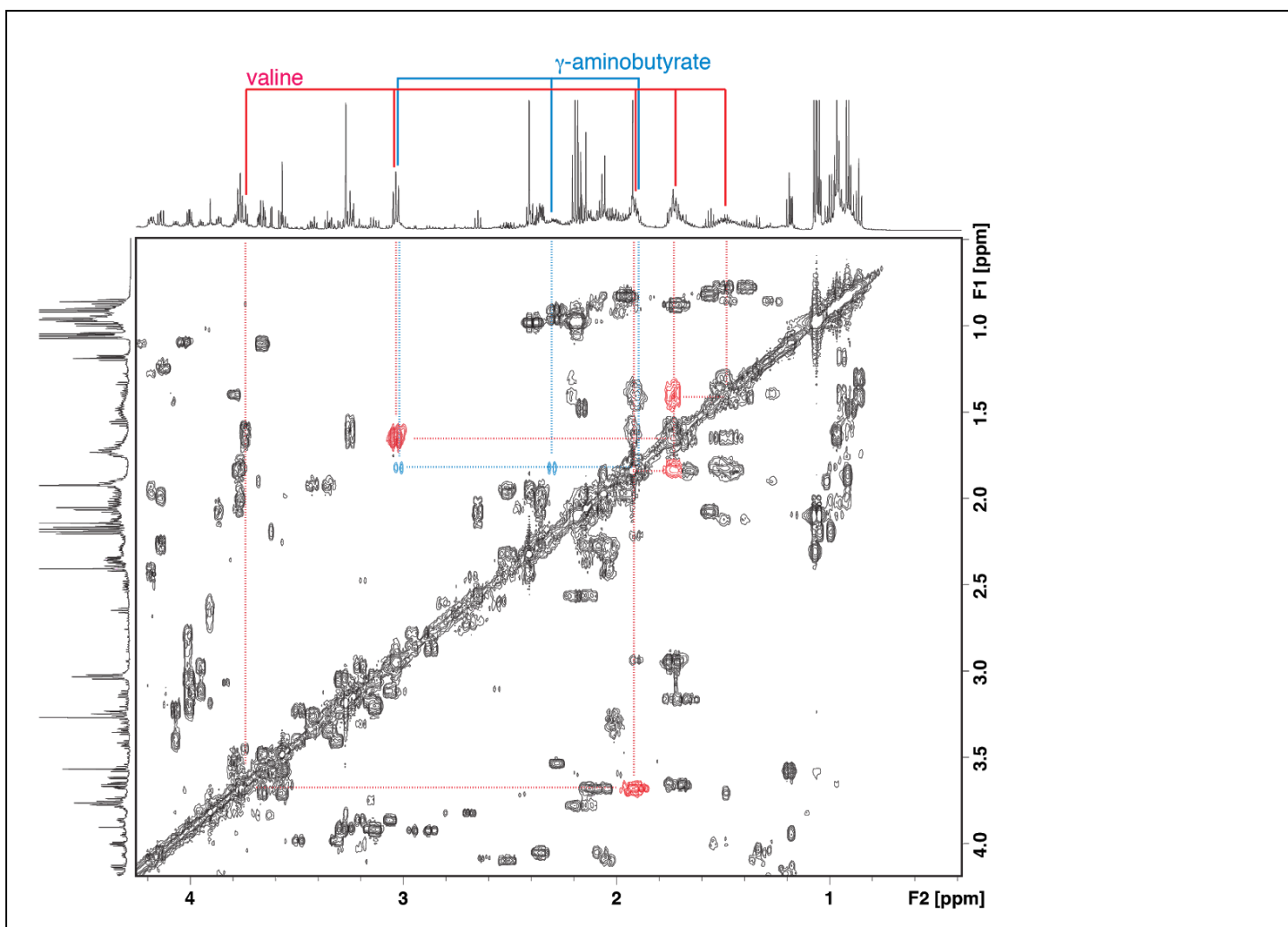


Figure S5. 2D ¹H NMR spectra collected on a single sample of supernatant from co-culture of *Clostridium* ASF356 and *Parabacteroides* ASF519 (related to STAR Methods, ¹H nuclear magnetic resonance spectroscopy-based metabolomics). 2D spectra show gamma-aminobutyrate, not 5-aminovalerate, in the region overlapping with valine in 1D. Peaks corresponding to valine shown in red, gamma-aminobutyrate shown in blue.

Table S1. Sequences for primers, probes, amplified products, and fluorophore and quencher pairs for each probe. Related to STAR

Methods, Hydrolysis probe-based qPCR assay design.

strain	Fluor/quencher	gene	forward	reverse (reverse complement)	full_product	probe
ASF356	Cy5/BHQ2	WP_04034147.1 ASF356	TGGTGCTGCAACAGAAA AACAGAAA CAG	GGTATTGTTGCTGGTGGTGG	TGGTGCTGCAACAGAAAACAG AAATGAAAGAGAAGAAATT AAGAATGGAAGATGCTTTAG CAGCTACAAGAGCAGCAGTA GAAGAAGGTATTGTTGCTGG TGGTGG	TGCTTTAGCAGCTAC AAGAGCAGCAGTAG A
ASF361	FAM/BHQ1	WP_04049972.1 ASF361	GTCGGATCTTAGGCCAC TAGGCCAC GAG	CAGTTGATGGGGGCATGAGT	GTCGGATCTTAGGCCACGAG GGGATCGGGATCGTCACTGA AGTTGCTAATGATGTAACCTT CGCTCAAAGTCGGTGATCGC GTAAGTATTGCTTGGTTCTTC AAGGGGTGTGGATACTGTGA ATACTGTACCACAGGTCGTG AAACTCTTTGTCGTAATGTA CAAAATGCAGGTTACTCAGT TGATGGGGGCATGAGT	ATCGGGATCGTCACT GAAGTTGCTAATGAT
ASF492	FAM/BHQ1	WP_04053549.1 ASF492	CAGTGGAAAGTCTGCA GCTCTGCA GGAA	GGGGATGAAGAGTCCGCAA	CAGTGGAAAGCTCTGCAGGAA ATGAGCCAGAAAGTAGACG GAAAAAATCACATTGCAAAA GTTGCGGCTATTTCCGCAGG GGATGAAGAAGTCGCAA	TCACATTGCAAAAAGT TGCGGCTATTTCCGC
ASF500	HEX/BHQ1	WP_023345698.1 ASF500	GGAAGTATCTCCGGGG CTCCGGGG GACT	TTGCGTGCTGTGTCAATGG	GGAAGTATCTCCGGGGGACT GGGCGGCGTATCTGCCGCTG GAGCGGGAAATCCCTAAAA ATGTCCGCAGAGCTATCGCT ATGGAGTGGTCACAGCTTCG AGAGGAAAATTCACCCTTGC GTGCTGTTGTCAATGG	TGCCGCTGGAGCGG GAAATCCCT
ASF502	TR/BHQ2	WP_04070354.1 ASF502	GCGAAGTCGTCGAAGG CGTCAAGG TACA	CCGACAGAGTGGCTCAGATC	GCGAAGTCCGTCAAGGTACA GAAGGAAAATACCGTAATCG TAGACGGCTCCGGCGACAAG CAGGCGATTGCCGACAGAGT GGCTCAGATC	AATCGTAGACGGCTC CGGCGACAAGCA
ASF519	HEX/BHQ1	WP_010802098.1 ASF519	CGCAGCTTCTATCGCTGG TATCGCTGG TA	CTCCGATGAACCCGGGTATG	CGCAGCTTCTATCGCTGGTA TGTTCTGACAACAGAATGT GTAATCGCAGAGAAGAAAG AAGATACTCCTGCTATGCCT CCGATGAACCCGGGTATG	TGTTCTGACAACAG AATGTGTAATCGCAG
ASF360	TR/BHQ2	WP_04039698.1 ASF360	AGCCGCTTTTGGATTG TTGGATTG GG	TGGTGGATCGGCGTAATTCC	AGCCGCTTTTGGATTGTTGG CTACAATTGCGCAAATTGGT CATAAGTCCGTCGTTTGCA TGATGCCAACTTTATCGGTT GGTGGTGGTGGATCGGCGTA ATTCC	AGTCCGTCGTTTGCA TGATGCCAACTTTAT

Table S3. Metabolites for which niche expansion was observed (related to Figure 6).

strains	metabolite
356,492	2-Oxoisocaproate
356,500	2-Oxoisocaproate
492,500	2-Oxoisocaproate
356,492	unknown26
356,500	unknown26
492,500	unknown26
360,361	Serine
361,500	unknown5
492,500	unknown5
356,492	Pyroglutamate
356,500	Pyroglutamate
492,500	Pyroglutamate
356,361	unknown25
356,361	Tryptophan
356,500	Tryptophan
361,500	Tryptophan
492,500	unknown16
360,492	unknown8
360,500	unknown8
492,500	unknown8
360,361	Leucine
360,492	Leucine
361,492	Leucine
361,500	Leucine
492,500	Leucine
361,500	Cystine
492,519	Nicotinamide
492,500	unknown2
500,519	Glycine
356,492	Phenylalanine
356,500	Phenylalanine
360,361	Phenylalanine
361,492	Phenylalanine
361,500	Phenylalanine
492,500	Phenylalanine
360,492	unknown12
360,500	unknown12
492,500	unknown12
492,500	unknown9
356,492	Aspartate
356,500	Aspartate
361,492	Aspartate
361,500	Aspartate
492,500	Aspartate
492,500	unknown7
356,500	Tyrosine
360,361	Threonine

360,500	Threonine
361,500	Threonine
361,500	unknown33
361,492	unknown3
361,500	unknown3
492,519	unknown4
361,500	Choline
360,492	unknown10
360,361	Succinate
361,492	Succinate
361,500	Succinate
492,500	Proline
492,500	Trehalose
492,500	unknown13
361,500	unknown1
492,500	Isoleucine
356,492	Methionine
356,500	Methionine
361,492	Propionate
361,500	Propionate
492,500	Propionate
361,500	Alanine
360,361	Valine
361,492	Valine
361,500	Valine
361,492	unknown18
356,492	Glutamate
361,492	Glutamate
492,500	UMP
356,492	Lysine
356,500	Lysine
360,361	Lysine
361,492	Lysine
361,500	Lysine
492,500	Lysine
492,500	Isocaproate
356,500	Asparagine
360,492	Asparagine
361,500	Asparagine
356,492	Isopropanol
356,500	Adenine
492,519	Cytosine
360,519	unknown32