**Material & Methods**

Microbial strains

Human gut bacterial strains of Blautia hydrogenotrophica S5a33 (DSM 10507T), Bacteroides thetaiotaomicron VPI-5482 (DSM 2079T) and Roseburia intestinalis L1-82 (DSM 14610T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany). The strains were frozen in Wilkins-Chalgren Anaerobe Broth (WC; Oxoid Ltd., Basingstoke, United Kingdom) plus 20% glycerol and maintained at -80°C until use.

Batch cultivation and sample collection

Batch cultivations of monoculture, bi-culture and tri-culture were followed for 120 h in 120-ml serum bottles containing 60 ml of WC medium. The serum bottles were prepared in a same way as previously described (*24*), and were inoculated with 1 ml of the diluted preculture to an OD600 of 0.1 (either a single species or the mixture of them). The bottles were incubated at 37 °C and at a constant stirring rate of 170 rpm (shaker KS 4000 i; IKA, Staufen, Germany). Samples were taken from the liquid broth every four hours for the first 48 h and every 12 h afterwards. Three biological replicates were designed for testing the monocultures in three independent batch experiments. All bi-culture and tri-culture experiments were performed in six biological replicates and always had a negative control bottle without inoculation but with sampling for each time, to verify its sterility.

Sterile syringes were used for each timepoint to collect 1 ml of the fermentation broth into 2-ml tubes (Eppendorf) under anoxic conditions. Subsequently, these tubes were used to measure OD600, pH, metabolites and to count bacterial cells by live/dead staining followed by flow cytometry.

Chemostat experiment with ambr 15

Fermentations were performed in the Ambr® 15 Fermentation (Ambr 15f) system (Sartorius Stedim Biotech, Royston, UK) located inside a Don Whitley A155 Anaerobic Workstation with HEPA filter (10% H2, 10% CO2, 80% N2, 55% humidity) as previously described (*27*). Strains reported were precultured first for 48 h, in modified Gifu Anaerobic Medium broth (mGAM, HyServe) then cultured for 18 hours in Wilkins-Chalgren anaerobe broth (WC, Oxoid, 1/100th dilution without washing) before inoculating the minibioreactors.

Prior to inoculation, strains were diluted in WC medium to an OD600 and mixed in even ratios and inoculated in the microbioreactors to a total volume of 10 ml (OD 0.001 for *Bacteroides thetaiotaomicron* and 0.002 for the others). A sample was taken at time point 0 and continuous feeding and sampling started at 4 hours after inoculation. The feed consisted of WC anaerobe broth and was deliver at an approximate rate of 0.04 h-1, resulting in a complete change of the medium in 24 hours. Samples (250 μl) were pipetted into a cooled plate (4°C). The supernatant was collected together in a separate plate and all three plates were stored at -80°C for further analysis.

In the first experiment, we first applied a pH perturbation by decreasing the pH of the feed (WC with pH 6.4 to pH 3.7) after 88 hours of growth for 32 hours. Subsequently, after 150 hours, we applied a perturbation in the dilution rate by stopping the feed for 15 hours (periodic removal of liquid continued), after which an additional 5 mL fresh medium (50%) was added to the vessels (refer to Supplementary movie S2).

The 16S rRNA gene of selected samples was sequenced (sample every eight hours in experiment 1 and every twelve hours in experiment 2).

Quantification of live cells with flow cytometry

We used a combination of the DNA-based stains SYBR Green I (SG; Invitrogen) and propidium iodide (PI; Invitrogen) to stain bacterial cells with intact and damaged cytoplasmic membranes (*35*). Under anoxic conditions, cells were diluted in filter-sterilized PBS buffer. 1:10 for the first two time points (0 and 4 h) and 1:200 for the next points and stained with a saturating solution of SG/PI, incubated for 20 min in the dark at 37 °C right and immediately measured by flow cytometry using the benchtop CytoFLEX S flow cytometer (Beckman Coulter, Brea, USA) instrument. Events were recorded for exactly 1 min at a sample flow rate of 10 μl/min, applying threshold values of 3000 and 2000 for the forward and side scatter, respectively, values that we have previously validated (*24*). We also used 0.5 μm and 1 μm green fluorescent beads (Thermo Fisher Scientific, USA) as internal standards. Raw flow cytometry data is deposited in flowrepository.org (IDs FR-FCM-Z6YM, FR-FCM-Z6YN, FR-FCM-Z74P, FR-FCM-Z753 and FR-FCM-Z754).

Flow cytometry data analysis

We used an in-house developed pipeline to accurately quantify the absolute abundance of live *Blautia hydrogenotrophica*, *Bacteroides thetaiotaomicron*, and *Roseburia intestinalis* cells. We clustered flow cytometry events in a UMAP space, following a detailed protocol available at: bit.ly/3WNrslL. Raw flow cytometry data were normalized, scaled, and transformed using the arcsin function. The data was then projected into three-dimensional UMAP space, allowing us to classify cell populations into four categories: “live,” “inviable,” “debris,” and “blank.” This classification was based on empirically determined thresholds for propidium iodide (PI) and SYBR green (SG) signals and by distinguishing from blank control runs.

Further analysis involved using monoculture samples for species classification in co-culture samples. We created a training space with labeled UMAP projections of random events from each monoculture replicate. Supervised UMAP and the K-nearest neighbors vote classifier (parameters: n\_neighbors = 50, weights = distance, and metric = Mahalanobis) were employed to assign species labels to co-culture events. Prior to finalizing classifications, each sample was overlaid with corresponding blank runs for manual verification using clickable three-dimensional scatter plots, ensuring accurate separation of cell populations from blanks. This process also validated our parameter choices for UMAP and the classifier. Additional details and scatter plot examples are available at: bit.ly/3WNrslL.

Metabolite profiling

After centrifuging the liquid broth for 20 min at 21,130 × g at 4 °C (Centrifuge 5424R; Eppendorf, Hamburg, Germany), the supernatants were used for measuring the concentrations of trehalose, glucose, pyruvate, succinate, formate, acetate, lactate and n-butyrate, which were determined in triplicate by high-performance liquid chromatography (HPLC) as previously reported (*24*). We also measured the first and end time points of the blank controls. Metabolites propionate, iso-butyrate, and isovalerate were measured but their concentrations were not consistently different from the blank WC control. Most of these metabolites are found in our system as organic acids. In the text, however, for brevity we refer to them by their salt names.

RNA extraction and sequencing

A total of 27 samples representing the different growth phases of monocultures in three biological replicates were selected for RNA sequencing, including timepoints of three independent experiments: monoculture *Blautia hydrogenotrophica* in WC (14h, 32h and 72h), monoculture *Bacteroides thetaitaomicron* in WC (4 h, 12 h and 36 h) and *Roseburia intestinalis* in WC (4 h, 12 h and 48 h). Details of the extraction and purification of total RNA, evaluation of RNA integrity and yield, as well as library preparation and sequencing can be found in our recent paper (*24*). Although we used the same methodology, the RNA sequencing data of *Blautia hydrogenotrophica* is unique to the current study. The raw RNA-seq data was deposited in the Sequence Read Archive (SAMN39333017-19 -https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1063153/; SAMN32321133-38, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA914119/).

RNA-seq data processing

Initially, low-quality reads and adapters were removed using fastp. We then mapped high-quality RNA reads to reference transcripts using Salmon in selective alignment mode, employing a decoy-aware index constructed from each organism’s genome. We used the latest reference genomes and transcripts from the BV-BRC database. from the counts files we used the R package DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html) to estimate the p-values between pairs of conditions. The R scripts for these analyses can be found here: https://github.com/danielriosgarza/hungerGamesModel/blob/main/scripts/R/

To integrate the gene expression data with genome annotations and metabolic information from the genome-scale metabolic models we wrote a Python class, which is available here: https://github.com/danielriosgarza/hungerGamesModel/blob/main/scripts/geneExpression/parseGenExpData.py. With this class one can, for example, draw the bar charts of Figure 1A, B, and C.

Modeling

In this manuscript we built two computational models based on ordinary differential equations: a mechanistic model based on metabolite and cells kinetic equations (depicted in Fig. 2A) and a phenomenological model based on the generalized Lotka Volterra dynamics (depicted in Fig. 4A). A detailed description of the model parameters, rationale, and experimental validation is available in the Supplementary Texts S1 and S2. A detailed implementation of the reported simulation and code to reproduce all of our computation analysis is available at the projects’s Github repository: https://github.com/danielriosgarza/hungerGamesModel, which also contains a comprehensive Wiki to help users reproduce our analysis, and detailed instructions to reproduce all the manuscript Figures in a Jupyter notebook (https://github.com/danielriosgarza/hungerGamesModel/blob/main/multistabilitymanuscript.ipynb).