Starvation response strategies impact the interaction dynamics of human gut bacteria: a study of *Bacteroides thetaiotaomicron* and *Roseburia intestinalis*

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Abstract

We explore the impact of nutrient scarcity on the interaction dynamics of two common human gut bacteria, *Bacteroides thetaiotaomicron* (BT) and *Roseburia intestinalis* (RI). Upon depletion of glucose, the viable cell numbers of BT decrease, whereas RI enters a slow growth mode. When mucin beads are supplemented, both species attach to the beads and the number of viable BT but not RI cells in liquid increases significantly. In co-culture, viable cell numbers decrease or increase significantly compared to monoculture, depending on the time point. A combination of targeted metabolomics and RNA-seq showed that RI's slow growth mode represents a diauxic shift towards acetate and lactate consumption, whereas BT survives glucose depletion by foraging mucin sugars. Most tested mucin monosaccharides inhibit the growth of RI but not BT on glucose. We encoded our findings in a kinetic model, which qualitatively reproduces the observed dynamics.

Introduction

The human gut is populated by hundreds of microbial species interacting with each other and with the human host. Gut bacterial species differ in their distribution along the human digestive tract and favor different microhabitats, such as the lumen of the large intestine, colonic crypts and mucus layers[1,2]. Diverse growth rates and attachment properties of gut bacteria help establish spatially structured communities. Also, the spatially heterogeneous environment influences the colonization success of different members of the gut microbiota and their competitive or cooperative relationships with one another[3–8].

An efficient way to acquire nutrients is essential for microbial persistence in the human gut. In addition to dietary fiber in the lumen, host-derived mucin glycans provide an alternative source of nutrients for members of the gut microbiota[9]. The colonic mucus layer, located at the interface between the epithelium and the gut microbiota, is key to maintain intestinal homeostasis and to enhance the resilience to perturbations of the gut microbiome[10,11]. Mucus is continuously secreted by goblet cells and is organized into two distinct layers: an inner dense layer tightly attached to the epithelium, which appears to be essentially sterile when healthy; and an outer loose layer with an expanded volume, which supports a metabolically diverse community. The outer layer provides mucin polysaccharides and

32 serves as attachment sites for the gut species that co-evolved to utilize these substrates,
33 giving rise to complex microbial interactions[9,10,12,13]. *Bacteroides* is one of the most
34 abundant but also most variable genera across human fecal samples[14]. Its most studied
35 species *Bacteroides thetaiotaomicron* (BT) holds a large repertoire of genes involved in
36 sensing and hydrolyzing many different polysaccharides, and BT was reported to strongly
37 express genes involved in the degradation of dietary plant glycans[15,16]. As a nutritional
38 generalist, BT can alter its metabolic response to mucin glycans in the absence of dietary
39 nutrients, to colonize the mucus layer and maintain its persistence in the human gut[17].
40
41 Short-chain fatty acids (SCFAs) including acetate, propionate and butyrate, are the major
42 end products of the anaerobic fermentation of polysaccharides by the human gut
43 microbiota. All these SCFAs exert multiple health benefits, in particular butyrate, which is
44 used preferentially as an energy source to fuel colonic enterocytes and may trigger signals
45 regulating mucin synthesis and secretion[18,19]. Metabolic cross-feeding of the human gut
46 microbiota impacts SCFA production[20]. For example, members of the most abundant
47 genera, including *Faecalibacterium*, *Eubacterium* and *Roseburia*, are able to convert acetate
48 and/or lactate into butyrate using the prevalent butyryl-CoA:acetate CoA-transferase
49 pathway[21,22]. Lactate is also an important end product of anaerobic fermentation in the
50 gut[23]. In addition to acetate as a key intermediate that promotes cross-feeding interactions
51 of the gut microbiota, the role of lactate as substrate for butyrate producers may explain its
52 low levels of accumulation in the human colon of healthy adults[24,25]. Upon exhaustion of the
53 primary carbon sources such as carbohydrates in the gut, microbes have to hunt for another
54 carbon source to thrive. It was reported that acetate could promote the growth of a
55 common intestinal butyrate-producing species *Roseburia intestinalis* (RI) during growth on
56 glucose[26–28].
57
58 Several studies employing in vitro fermentation systems have shown mucin as a driving
59 force in forming a microbial community that is phylogenetically and metabolically distinct
60 from the luminal one[29,30]. 16S rRNA gene-based results of complex communities from an in
61 vitro gut model showed that RI colonized mucin-covered carriers[31]. For unclear reasons,
62 *Roseburia* but not *Bacteroides* decreased in relative abundance with mucin
63 supplementation[32]. We thus require a mechanistic understanding of how mucin formed

microhabitats impact the abundance of different gut species. Here, we built an in vitro batch system containing mucin beads, to systematically investigate how a spatially structured environment in the presence of mucin beads influences the growth and interactions of the model gut species BT and RI. The growth dynamics of monocultures and co-cultures in the liquid medium and attached to the beads were monitored using live/dead cell staining followed by flow cytometry in combination with machine learning techniques to quantify their proportions in co-culture. Subsequently, we characterized the effect of mucin degradation products on the abundance of BT and RI, and found that it depended on the sugar level being considered. Transcriptomic analyses of monoculture and co-culture in medium with and without mucin beads at several time points revealed different nutrient harvesting strategies employed by BT and RI, which resulted in their different growth modes, and also changed their interaction mechanisms depending on nutrient availability. We further developed a kinetic model that accounts for pH conditions, metabolites, and growth profiles, to mechanistically understand how gel-entrapped mucin influences the proliferation of BT and RI and the dynamics of their relationship.

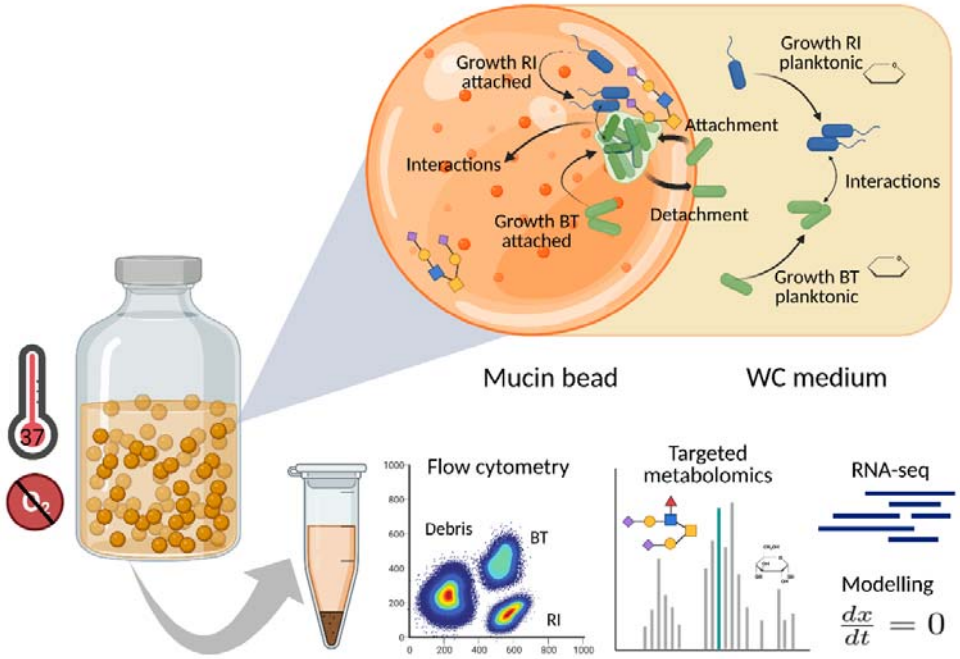

Figure 1. Experimental setup and design. We used a bottle system containing mucin beads in WC medium to mimic the spatially structured environment of the human gut. The growth dynamics of monoculture and co-culture in the liquid medium and attached to the beads

were monitored using flow cytometry with live/dead cell staining. Live cells of BT and RI were differentiated from each other and from debris using a UMAP-based classification strategy (details in the "Methods" section). Fermentation products, including short-chain fatty acids and mucin-derived monomeric sugars, were measured by high-performance liquid chromatography and gas chromatography. The presence and quantity of mRNA in samples collected at different time points were assessed by RNA-seq. Further, a kinetic model with ordinary differential equations was developed that summarizes our understanding of the system. O_2: oxygen; WC: Wilkins Chalgren medium; BT: *Bacteroides thetaiotaomicron*; RI: *Roseburia intestinalis*.

Results

Different responses to sugar depletion

Glucose and pyruvate were provided as the main carbon sources in WC (Wilkins Chalgren) medium (Figure 2a). When grown in monoculture, BT produced succinate, lactate, formate, and acetate during fermentation, which decreased the pH from 6.7 to 5.0. At 16 h, BT reached its maximal density of 1.2×10^6 viable cells/µl. After the depletion of glucose and pyruvate, the acidic conditions led to a loss of viable BT cells but did not affect the total cell count. Loss of viability at low pH (5.0) was further confirmed by spent media and pH experiments. We re-grew BT in its spent medium (collected at 48 h cultivation when glucose is depleted), and found that an initial pH of 6.5 is required for growth (on trehalose; Figure S1). Supplementing the spent medium with glucose and pyruvate only enhanced growth when the pH was also increased (Figure S1a). In addition, BT did not grow on glucose and pyruvate when the pH of WC medium was lowered to 4.5 and 5.0, respectively, and its growth rate was decreased at pH 5.5 and 6.0 compared to pH 6.7 (Figure S1b). RI in monoculture produced lactate, formate, acetate and butyrate from glucose and pyruvate, corresponding to a pH decrease from 6.7 to 5.7 at 16 h and reached a maximal density of 7.8 $\times 10^5$ viable cells/µl (Figure 2b). After the depletion of glucose and pyruvate in 24 h, RI gradually entered a slow growth mode, seen as a drop in viable cells to 1.8-3.8 $\times 10^5$ cells/µl from 40 h to 120 h in two independent experiments. In addition, we observed a further significant ($P < 0.001$ by Student's t test) increase of butyrate concentration concurrent with decreasing acetate and, in some cases, lactate (Figure 2b and Figure S2 for lactate

115 consumption in RI monoculture), resulting in a pH increase from 5.7 to 6.0. In brief, viable
116 BT cells decline primarily due to the lowered pH, whereas RI maintains higher cell densities
117 by entering a slow growth mode.
118
119 Given the observed diauxic shift from glucose/pyruvate to lactate/acetate, we performed
120 RNA-seq of RI grown in monoculture for several time points and replicates to investigate its
121 metabolic mechanism. During the exponential growth period, genes associated with
122 glycolytic and pyruvate-utilizing pathways were more highly expressed at mid-log phase of
123 12 h compared to at 4 h (Figure S3b). Interestingly, although glucose ran out at 48 h, we
124 found that RI kept expressing glycolysis genes and also non-essential genes such as those
125 involved in cell motility although growing in a well-mixed environment (Figure S4). We
126 further compared the effect of different normalization methods (transcripts per million and
127 DESeq2's median of ratios[33]). In general, both methods showed consistent results except for
128 some glycolysis genes, including hexokinase (HEX1; RIL182_00253) and phosphoglycerate
129 mutase (PGM; RIL182_01289) (Figure S5). We used a strict cutoff of at least 1.5-fold change
130 and a Benjamini-Hochberg adjusted *P* value less than or equal to 0.01 for differential
131 expression calculated by DESeq2. When comparing the transcript levels of RI in monoculture
132 between 12 h and 48 h, the significant up-regulation of genes encoding lactate
133 dehydrogenase (RIL182_00335 and 01719) and butyryl-CoA:acetate CoA-transferase
134 (RIL182_00413) corroborates the finding that RI converts lactate/acetate to butyrate after
135 glucose depletion. The energy yielded by butyrate production from lactate/acetate ($\Delta_f G^0$ = -
136 46 KJ/mol) is much lower than that from glucose ($\Delta_f G^0$ = -250 KJ/mol)[34]. Therefore, it is not
137 surprising that RI grows more slowly on lactate/acetate. In summary, RI maintains its slow
138 growth in the absence of glucose by converting acetate and lactate to butyrate.
139
140 **Effects of mucin on monoculture**
141 BT survives longer in the presence of mucin beads than without them. After depleting
142 glucose and pyruvate at 24 h, BT in liquid retained a viable cell count higher than 1.0×10^6
143 cells/μl (Figure 2d). We further extended the cultivation period with mucin beads from 120
144 h to 288 h and observed a consistently stable population of BT in the liquid broth (Figure
145 S6). In addition, the growth of RI on glucose/pyruvate seems to be impeded by mucin,
146 demonstrated by a longer lag phase of 12 h and lower maximum of 4.5×10^5 viable cells/μl

147 at 36 h when compared with RI grown in WC without mucin beads (Figure 2e). After nutrient
148 depletion, RI still exhibited the slow growth mode characterized by a further increase of
149 butyrate and decrease of lactate/acetate, but with a much lower density of 0.3-0.5 × 10^5
150 viable cells/μl than in the absence of mucin. In addition to the planktonic cells in liquid, we
151 also quantified the cells of BT and RI attached to the mucin beads at different time points.
152 As shown in Figure 2d and 2e, attached BT and RI in monoculture reached maximal densities
153 of 6.0 × 10^5 viable cells/μl at 24 h and 1.1 × 10^5 viable cells/μl at 32 h, respectively. Thus, in
154 the presence of mucin, BT can maintain high densities in the long term, whereas the density
155 of RI declines.
156
157 Regarding the mucin degradation products, the concentration of *N*-Acetylneuraminic acid
158 (Neu5Ac) showed a significant ($P < 0.001$ by Student's t test) increase of 0.12 mM in BT
159 monoculture after 120 h and no significant change in RI monoculture (Figure 2d-e). We also
160 observed higher levels of fucose and galactose for BT grown with mucin beads than without
161 it. The dynamics of galactose in RI monoculture indicates that RI also hydrolyzed mucin and
162 further utilized its degradation products such as galactose. To investigate the effects of
163 mucin degradation products on the growth of BT and RI at different glucose levels,
164 monosaccharides including Neu5Ac, *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose,
165 galactose, mannose, and their mixture were added to the standard WC medium and WC
166 medium without glucose and pyruvate in 24-well plates. In the presence of
167 glucose/pyruvate, none of the supplemented sugars influenced BT growth significantly ($P >$
168 0.05 by Student's t test) compared to the WC control (Figure S7a). In contrast, Neu5Ac
169 impacted RI growth positively while other sugars had negative effects (Figure S7c). In the
170 absence of glucose/pyruvate, all sugars except Neu5Ac had positive effects on BT growth
171 (Figure S7b), and all sugars, in particular galactose, promoted RI growth (Figure S7d). In
172 conclusion, mucin-derived monosaccharides have species-specific effects that in addition
173 depend on the level of glucose.
174
175 The RNA-seq data further elucidated the different responses of BT and RI to mucin. For BT,
176 we first collected a list of genes/operons associated with the polysaccharide utilization loci
177 of mucin *O*-glycans, which were possibly activated by two types of transcriptional
178 regulators, namely extracytoplasmic function sigma/anti-sigma pair (ECF-σ) and hybrid two-

179 component system (HTCS)[35]. Interestingly, those genes linked to HTCS and ECF-σ showed
180 significantly high expression in the lag phase of 4 h (Figure 3a; $P < 0.001$ for both by
181 Student's t test), and genes of ECF-σ-linked loci were also expressed after the
182 glucose/pyruvate depletion (48 h and 120 h), indicating BT utilization of mucin *O*-glycan
183 components, which further supports the hypothesis that nutrients derived from mucin were
184 responsible for the long-term survival of BT. Specifically, after glucose/pyruvate ran out at
185 24 h, we observed a significantly ($P < 0.001$ in all cases; Student's t test) increased
186 expression of genes encoding the extracellular galactosidase (BT_3340, 4684) and
187 fucosidase (BT_1842), as well as genes involved in the utilization of galactose (BT_0370-71,
188 0623) and fucose (BT_3606, 1273-75). These results are consistent with BT metabolism of
189 mucin *O*-glycans including galactose and fucose (Figures 2d and S7b). Based on expression
190 levels measured in transcripts per million, RI consistently increased the expression of genes
191 involved in glycolysis, pyruvate utilization, mucin hydrolysis, galactose and mannose
192 utilization, and the butyrate-producing process after depletion of glucose/pyruvate, with or
193 without mucin beads present in the medium (Figures 3b and S3b). At a high
194 glucose/pyruvate level (Figure S8a), the presence of mucin significantly reduced the
195 expression of genes associated with the transport of glucose (RIL182_03793, 03794, 03796),
196 pyruvate (RIL182_01689), galactose (RIL182_01067-69) and *N*-acetylglucosamine
197 (RIL182_02688-89) across the outer membrane. At a low glucose/pyruvate level, our results
198 suggest that mucin impaired the utilization of lactate and acetate. For example, genes of
199 lactate dehydrogenase (RIL182_00335) and butyryl-CoA:acetate CoA-transferase
200 (RIL182_00413) were significantly down-regulated (Figure S8b). Thus, mucin supports the
201 long-term survival of BT, while it undermines RI's growth on glucose/pyruvate and
202 lactate/acetate.
203
204 **Impact of mucin on interaction dynamics**
205 For the co-culture grown in WC, the co-utilization of glucose and pyruvate resulted in two
206 separate density peaks of 7.4 and 4.6 × 10^5 viable cells/µl for BT and RI, respectively (Figure
207 2c). The pH decreased from 6.7 to 5.3, which was not as acidic as in the BT monoculture
208 (5.0) but lower than in RI monoculture (5.7). After depletion of glucose and pyruvate, BT's
209 viable cell numbers gradually went down, as in monoculture. Interestingly, RI presented a
210 second peak of 4.1 × 10^5 viable cells/µl at 38 h. In addition, the net concentration of lactate

211 in co-culture was significantly lower than in each monoculture ($P < 0.001$ in both cases;
212 Student's t test). For the co-culture grown in the presence of mucin beads, BT reached a
213 maximal density of 1.5×10^6 viable cells/µl at 16 h, which was two-fold higher than that in
214 WC. RI in the mucin co-culture exhibited significantly lower cell densities than in WC co-
215 culture ($P < 0.01$ by Student's t test), accompanied by a lower maximal density of 1.8×10^5
216 viable cells/µl. The pH of 5.1 was nearly as acidic as in the BT monoculture with or without
217 mucin. A second peak of RI density in mucin co-culture was observed at 120 h,
218 corresponding to a roughly ten-fold increase in density from 96 h to 120 h (Figure S6; mean
219 values of three independent experiments with three biological replicates). The significant (P
220 < 0.001 by Student's t test) increase of galactose in the co-culture compared to the BT
221 monoculture suggests that both species hydrolyzed mucin (Figure 2f).
222
223 To investigate the interaction dynamics of BT and RI and mucin's impact on it, we compared
224 viable cell counts in co-culture and monoculture over time. We computed the interaction
225 strength, here defined as the impact of one organism on the other's abundance, as the log
226 ratio of viable cell counts in liquid in co- versus monocultures. As shown in Figure S9, the
227 interaction strength and in some cases its sign (i.e., positive or negative impact) changed
228 over time for both organisms with and without mucin beads. In the presence of
229 glucose/pyruvate, the competition for these nutrients resulted in negative interaction
230 strengths for both organisms. After depletion of glucose/pyruvate and in the absence of
231 mucin, RI impacts BT positively, likely by increasing the pH in the co-culture compared to the
232 BT monoculture. In the presence of mucin, BT's impact on RI during the initial growth on
233 glucose/pyruvate (20-48 h) changed from negative to positive, likely by reducing the
234 concentration of mucin degradation products that affect RI's growth negatively in the
235 presence of glucose. In summary, changing conditions alter interaction mechanisms
236 between the two species, which lead to varying interaction signs and strengths.
237
238 To further elucidate their changing interaction mechanisms across growth phases, we
239 compared the differential gene expression between co-culture and monoculture (Figure 3c-
240 d). In the presence of RI, BT genes associated with the transport of glucose (BT_0371, 1758)
241 were significantly down-regulated in WC without mucin (Figure 3c; I, II). In addition, the
242 expression of six out of ten glycolysis genes as well as genes involved in succinate, acetate

243 and formate production was significantly lowered in WC at low glucose/pyruvate levels
244 (Figure 3c; II). With mucin, BT up-regulated glycolysis genes and genes involved in producing
245 formate, acetate, succinate and lactate (Figure 3c; III, IV). At low nutrient levels, it also up-
246 regulated genes encoding the exocellular galactosidase (BT_3340, 4684), fucosidase
247 (BT_1842), mannosidase (BT_0458) and sialidase (BT_0455) in response to mucin hydrolysis,
248 and genes associated with the utilization of galactose and *N*-Acetylgalactosamine (Figure 3c;
249 IV). BT genes (BT_0617-22) belonging to the membrane-associated, proton-translocating
250 ferredoxin:NAD^+ oxidoreductase (*Rnf* complex) were also more expressed with mucin
251 (Figure 3c; III, IV). In conclusion, BT down-regulates the genes of glycolysis and pyruvate
252 oxidation when RI is present in WC, but does the opposite in WC and mucin.
253
254 In the presence of BT, RI genes associated with glucose transport (RIL182_03793-94, 03796,
255 01587-88) and the ATP generation via proton pump (RIL182_00203-10) were down-
256 regulated at high and low glucose/pyruvate levels, respectively (Figure 3d; I and II,
257 respectively). A candidate gene of the alarmone ppGpp biosynthesis was significantly up-
258 regulated (RIL182_00174). It is part of RI's response to stress such as carbon starvation and
259 may be involved in triggering its diauxic shift. RI genes associated with the import of glucose
260 (RIL182_03794, 03796), pyruvate (RIL182_01689), galactose (RIL182_01067-69) and *N*-
261 acetylglucosamine (RIL182_02688-89), as well as seven out of ten glycolysis genes were
262 systematically down-regulated at high glucose/pyruvate levels with mucin (Figure 3d; III). In
263 addition, RI genes associated with the butyrate-producing process via butyryl-CoA:acetate
264 CoA-transferase pathway were more expressed when glucose/pyruvate was depleted, with
265 or without mucin (Figure 3d; II, IV). BT restricts RI's import of glucose and assists its
266 production of butyrate from lactate/acetate, regardless of mucin supplementation.

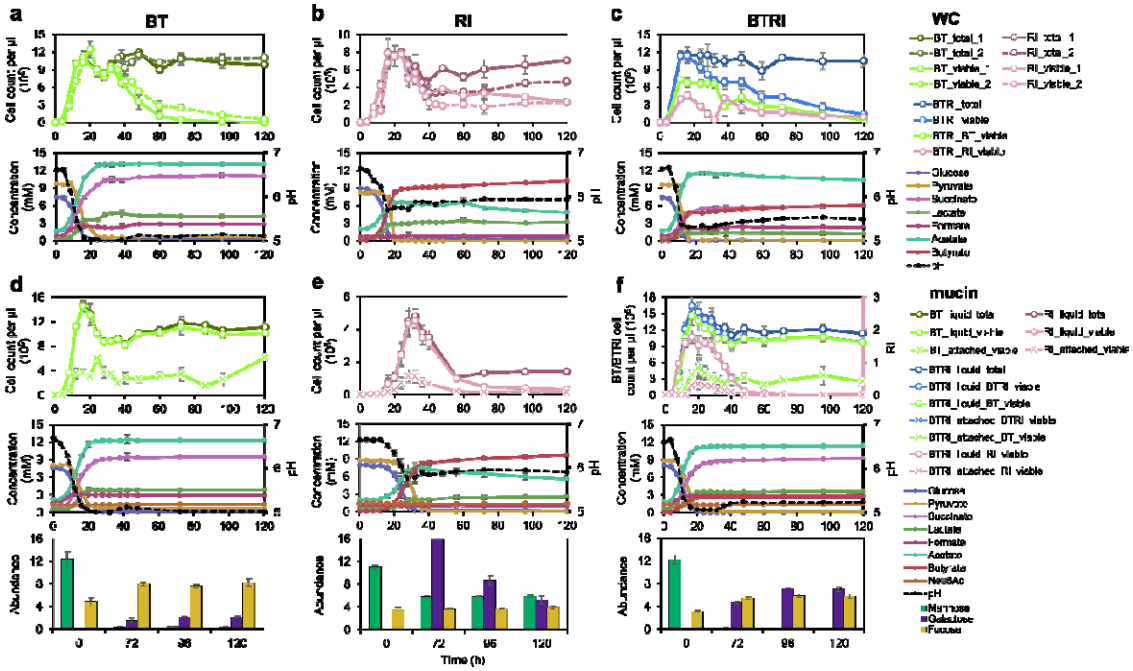

Figure 2. Performance of BT and RI in monoculture and co-culture without and with mucin beads during batch fermentation. Cell density, concentrations of glucose, pyruvate and fermentation products, and pH values for BT monoculture **(a)**, RI monoculture **(b)** and their co-culture **(c)** in WC medium during 120 h cultivation; Cell density, concentrations of glucose, pyruvate and fermentation products, pH values as well as concentrations of mucin monomeric sugars for BT monoculture **(d)**, RI monoculture **(e)** and their co-culture **(f)** in WC with mucin beads during 120 h cultivation. Circles represent the mean values of biological replicates. Error bars indicate the standard deviation of biological replicates (n=3 for BT and RI monocultures in WC experiment 1; n=2 for BT and RI monocultures in WC experiment 2; n=6 for co-culture in WC; n=3 and n=5 for BT and RI monocultures in WC with mucin beads, respectively; n=5 for co-culture in WC with mucin beads). Concentrations of mucin sugars are represented by the integrated area under the peak corresponding to the metabolite. To show the same range on the y axis of barplots, we divided these values by factors of 1,000,000, 5,000,000 and 10,000 for galactose, mannose and fucose, respectively. WC: Wilkins Chalgren medium; BT: *Bacteroides thetaiotaomicron;* RI: *Roseburia intestinalis*; Neu5Ac: *N*-Acetylneuraminic acid.

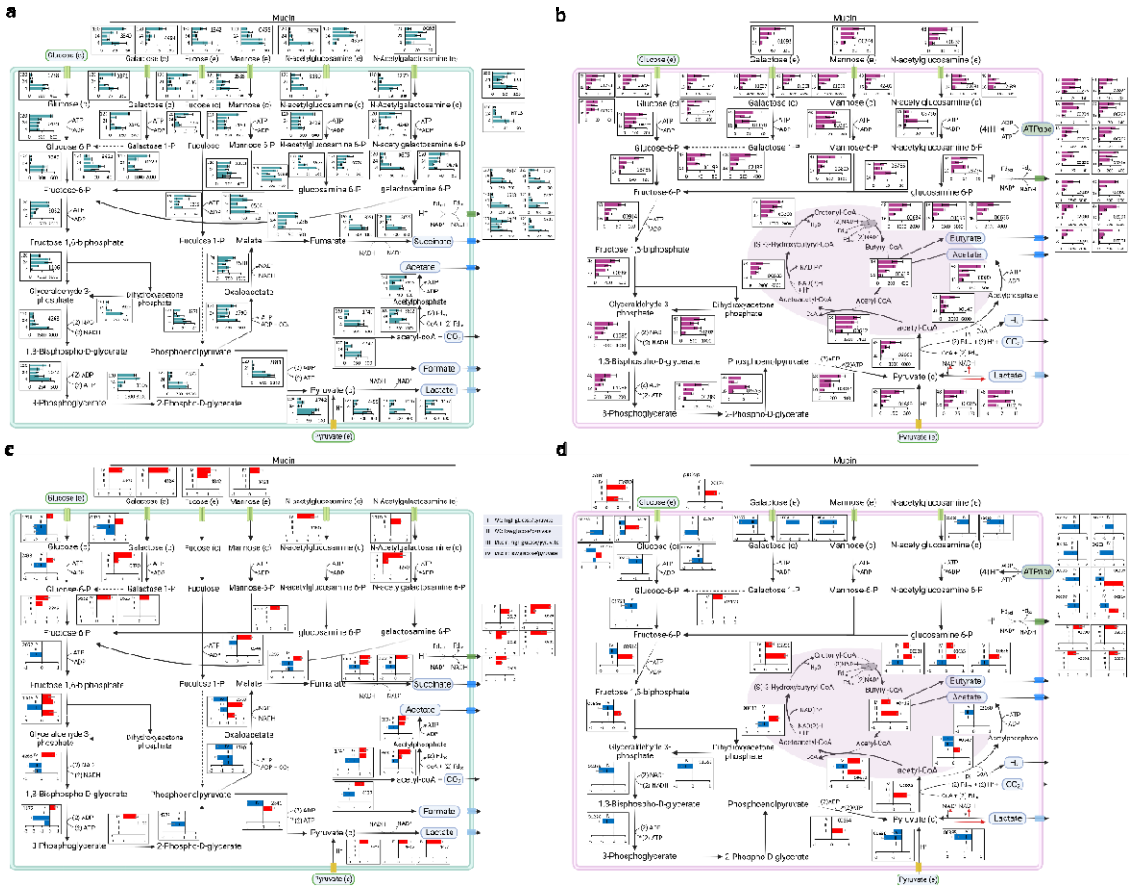

Figure 3. Metabolic maps summarizing gene expression in monocultures and significantly differential expression in co- versus monocultures of BT and RI. Gene expression of the key metabolic routes of glucose and pyruvate fermentation, as well as mucin hydrolysis and mucin sugars utilization in BT **(a)** and RI **(b)** monocultures in WC medium with mucin beads (Supplementary files 3 and 4); differential gene expression between co-culture and monocultures of BT **(c)** and RI **(d)**. In each barplot of **a** and **b**, gene expression levels (x axis) were measured in transcripts per million of RNA samples at time (y axis) 4 h, 12 h, 24 h, 48 h and 120 h for BT, and at time 16 h, 28 h, 40 h and 48 h for RI. In each barplot of **c** and **d**, differential gene expression was calculated with DESeq2 for four categories: high levels of glucose/pyruvate in WC (I), low levels of glucose/pyruvate in WC (II), high levels of glucose/pyruvate in WC with mucin beads (III) and low levels of glucose/pyruvate in WC with mucin beads (IV); positive and negative values of $\log_2$fold-change are shown in red and blue on the x axis, respectively. Numbers in each barplot stand for the Gene ID in the genomes of BT and RI. The solid and dashed lines represent single- and multi-enzyme

301 reactions between the two indicated molecules, respectively. Error bars indicate the
302 standard deviation of three biological replicates. Metabolic maps with gene expression in BT
303 and RI monocultures in WC are shown in Figure S3 and the Supplementary files 3 and 4. ECF:
304 extracytoplasmic function sigma/anti-sigma pair; HTCS: hybrid two-component system;
305 GlcR: HTH-type transcriptional repressor of glucose; ppGpp: alarmone guanosine-5'-3'-
306 bispyrophosphate.
307
308 **Kinetic model reproduces the observed dynamics**
309 To reproduce physiological observables such as total and viable cell counts as well as
310 concentrations of key nutrients and metabolites, we developed a model with ordinary
311 differential equations that summarizes our understanding of the interaction mechanisms
312 (Figure 4a). Since we see a roughly linear relationship between counts of viable attached
313 and free-swimming cells (Figure S10), the model assumes that a fixed fraction of the cells
314 attaches to mucin beads and that only these cells can degrade mucin. Mucin is treated as an
315 inexhaustible resource. We further assume that changes in SCFAs fully determine changes in
316 the pH, since we observed a strong linear relationship between pH and the sum of the
317 concentrations of SCFAs (Figure S11). Based on the literature, we also assume that a low pH
318 affects the growth of BT more strongly than that of RI, i.e., BT is more sensitive to acidity[36].
319 In addition, BT and RI can both grow on glucose and mucin sugars, which was well-
320 supported by our fermentation data and also confirmed at the transcriptional level. Given
321 our microtiter plate data, we further assume that mucin degradation products inhibit RI's
322 growth on glucose (Figure S7c). RI only switches to its lactate/acetate-consuming slow
323 growth mode when the concentrations of glucose and mucin degradation products are low
324 (Figure S8b). Finally, we hypothesize that glucose inhibits BT's growth on mucin sugars,
325 which is supported by the significant overexpression of the cAMP-Crp gene in the presence
326 of glucose ($\log_2$fold-change of BT_2187 in mucin monoculture: 1.7 for 4 h vs 12 h, 3.6 for 4 h
327 vs 24 h and 1.8 for 12 h vs 24 h). We simplified the model by treating glucose and pyruvate,
328 acetate and lactate as well as mucin sugars as single entities (see model scheme in Figure
329 4a). The slow growth mode of RI and the behavior of BT on mucin after depletion of sugar
330 are represented by separate states (in both cases referred to as 'slow growth mode').
331 Parameter values including metabolite production and consumption as well as state

switching rates were determined manually. The equations, parameter values and initial conditions are given in the Supplementary file 2.

The model qualitatively reproduces our observations, including the second peak observed for BT at 32 h in WC monoculture, the long-term survival of RI in WC and of BT in the presence of mucin beads (Figure 4b). However, the model fails to reproduce the second peak for RI in WC in co-culture, implying that additional mechanisms are at play that are not yet fully understood.

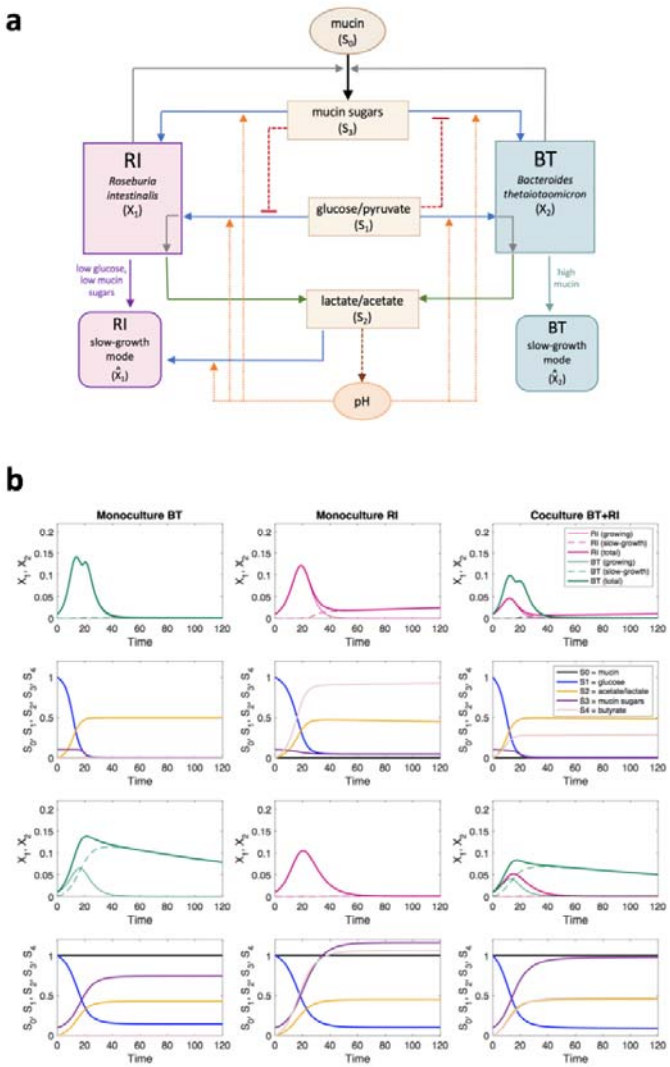

Figure 4. Model scheme and simulations reproducing observations qualitatively. a, Scheme of the kinetic model. **b**, Dynamics generated by the model without and with mucin. The equations, parameter values and initial conditions are given in Supplementary file 2.

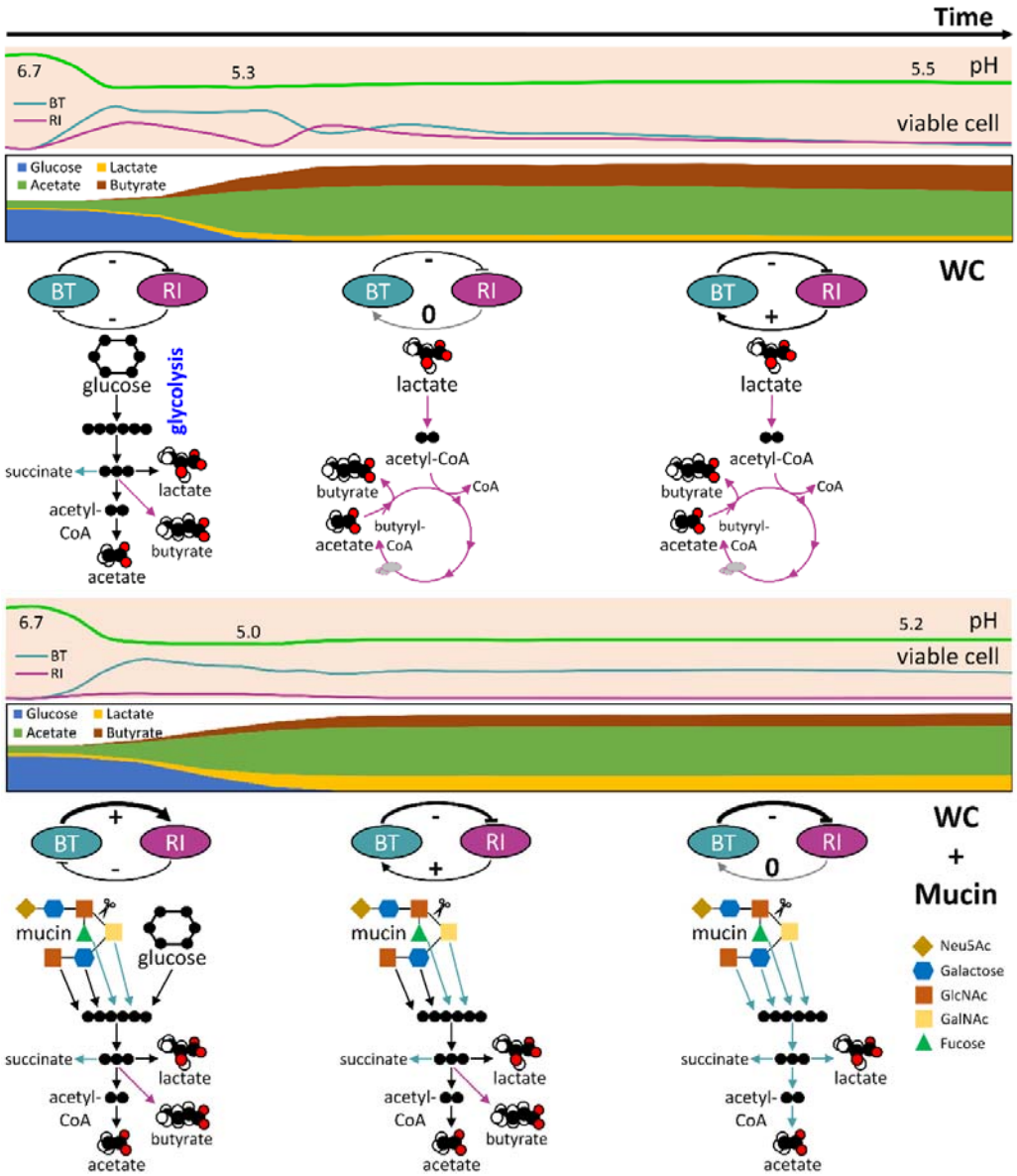

Figure 5. Summary of the interaction mechanisms driving abundances and metabolite concentrations in the BT and RI co-cultures without and with mucin beads. Signs of positive, negative and absence of an interaction are shown as +, - and 0, respectively. The thickness of the arrow represents the interaction strength quantified as the logarithm of viable cell density ratios in co- versus monocultures (detailed in Figures S9 and S12). Main metabolic pathways including glycolysis, converting lactate/acetate to butyrate, mucin hydrolysis and mucin sugars utilization are colored in teal, pink and black for BT, RI and both, respectively. Neu5Ac: *N*-acetylneuraminic acid; GlcNAc: *N*-acetylglucosamine; GalNAc: *N*-acetylgalactosamine.

356 **Discussion**

357 Here, we systematically investigated the molecular mechanisms underlying the ecological
358 interactions between two representative gut bacteria in the presence of simple and
359 complex carbon sources within a spatially structured environment (Figure 5). Our results are
360 in agreement with known niches in the human gut: BT survives as a mucus resident and RI
361 prefers to stay in the lumen[17,32].
362
363 We found that even when feeding on similar resources, BT and RI used alternative strategies
364 to cope with and anticipate nutrient scarcity. Growth data combined with temporal gene
365 expression patterns suggest that BT acts as a 'funnel' with its central carbon/energy
366 metabolism capable of tapping several monosaccharides. Independent entry points are
367 funneled into glycolysis until phosphoenolpyruvate is produced, which is converted into
368 oxaloacetate by fixing a CO_2 molecule with the generation of an extra ATP[37]. CO_2 could be
369 supplied from either the external environment or the conversion of pyruvate to acetyl-CoA.
370 Although this strategy results in accelerated growth, it comes at the cost of significantly
371 inferior performance when actively consumed nutrients are depleted. In contrast, RI uses
372 carbon chain elongation to generate additional energy and maintain a redox balance (Figure
373 3b), resulting in butyrate production. Although its growth is slower than that of BT, it can
374 switch its energy source to acetate and lactate, which are also produced by BT. This diauxic
375 shift resembles the nutrient utilization strategies adopted by other bacterial species[38],
376 mostly well characterized in *E. coli* [39–42]. Some butyrate producers such as RI can overcome
377 the energetic barrier to convert lactate to pyruvate by employing the flavin-based electron
378 confurcation: driving electron flow from lactate to NAD^+ at the cost of exergonic electron
379 flow from reduced ferredoxin to NAD^+. However, producing butyrate from lactate and
380 acetate is a much lower energy-yielding process, compared to glucose[34]. Slow growth is also
381 a known strategy to deal with starvation in other nonsporulating bacteria[43].
382
383 BT's growth strategy results in a dramatic reduction in the environmental pH, which
384 significantly inhibits its own growth. This self-inhibition resembles the hypothesis of
385 ecological suicide suggested by Ratzke et al. with some additional complexity[44]. At low pH,
386 BT cells remain viable for an extended period of time when mucin beads are present.

387 Without mucin, when glucose and pyruvate are depleted, a loss of viability is observed
388 throughout the population (Figure 2a). The depletion of nutrients per se is probably not the
389 cause of this loss since when the pH was restored in a spent medium assay, the BT cells
390 metabolized an alternative carbon source and restored growth (Figure S1). Taken together,
391 these results suggest that similar to other bacteria[45], BT requires an energy source to be
392 metabolically active in an acidic environment, possibly channeling its ATP from growth to
393 maintaining its intracellular pH within a physiologically viable range. When this source of
394 energy is no longer available, cells quickly lose viability and no longer have the opportunity
395 to utilize new nutrients. Interestingly, pH also opens up a new dimension in the ecological
396 interactions between BT and RI, particularly in WC medium. As shown in Figure 5, although
397 the two species compete for glucose and pyruvate, and thus initially sustain a negative
398 interaction, the glucose and pyruvate that RI manages to consume result in a smaller
399 decrease in the environmental pH (since RI does not lower the pH as much as BT), leading to
400 an indirect positive effect on BT at later stages.
401
402 We highlighted the asymmetries in the way BT and RI respond to mucin, whose positive or
403 negative effect depends on the presence or absence of glucose during fermentation. In the
404 absence of glucose, most of the mucin sugars tested boost BT's growth, except for Neu5Ac.
405 Analysis of its genome reveals the absence of a Neu5Ac lyase (*nanL*) gene. BT is unable to
406 catabolize Neu5Ac and did not grow on it in a minimal medium[46,47]. As mucin beads and the
407 tested mucin sugars did not significantly affect BT's growth during glucose fermentation
408 (Figure S7a), we assume that glucose prevents BT from simultaneously consuming other
409 carbon sources. This assumption was also necessary to reproduce BT's behavior with our
410 model. In *E. coli*, further uptake of carbon sources is suppressed by the transcriptional
411 regulator cAMP-Crp when nutrient uptake surpasses anabolism[38]. Here, we found that a
412 candidate gene of cAMP-Crp (BT_2187) was significantly over-expressed when BT consumed
413 glucose. As BT was reported to easily switch its metabolism from dietary input to mucin
414 glycans[17], we assume that BT relies on cAMP-Crp regulation to cope with excessive carbon
415 flux. In contrast to BT, RI does not grow well when glucose and mucin are supplied together
416 (Figure 2e). Adding mucin beads also significantly up-regulated the gene of HTH-type
417 transcriptional repressor GlcR (RIL182_03930), which indicated the repression of glucose
418 catabolism by RI. As no cAMP-Crp genes were found in its genome, we assume that glucose

419 and mucin sugars may compete for ATP-binding cassette transporters. Combined with
420 slower growth on mucin sugars, this may explain the negative effects of mucin sugars on RI's
421 growth on glucose. This is also supported by its significantly increased abundance in the
422 mucin co-culture (Figure 5), which likely resulted from a decreased concentration of mucin
423 sugars such as mannose due to uptake by BT. However, a mechanism of RI's carbon uptake
424 strategy has to be explored further.
425
426 Glycan-mediated attachment was reported as an important strategy for gut bacteria to
427 access nutrients[8]. Since both BT and RI attached to the mucin beads, the physical proximity
428 could influence their interactions on the beads. The beads were also not saturated by cells,
429 illustrated by a roughly linear relationship between density on beads and in the liquid of co-
430 culture (Figure S13). In addition, the observed fast attachment and detachment implies that
431 the dynamics between attached and free-swimming cells may be tightly coupled. Spatial
432 transcriptomics at a single-cell resolution may further reveal the interaction mechanisms of
433 human gut bacteria in a spatial context[48].
434
435 In the presence of BT, RI exhibited additional density peaks at 38 h without mucin and at
436 120 h with mucin, which were not predicted by our model. In most of the cases, the
437 classification technique we used showed high accuracy in distinguishing BT and RI on a basis
438 of their optical characteristics in flow cytometry, and the method accuracy was confirmed
439 with the data of a GFP labelled BT (Figure S14). However, when the cells are losing viability
440 (Figure 2c, 24-38 h), the similarity between live and inviable cells causes difficulties in
441 species separation. Thus, the transition from the first peak of viable RI cells to the second in
442 the WC co-culture may be smoother than observed. There are also alternative explanations
443 for the peak. Since BT can produce a higher concentration of lactate than RI in monoculture,
444 a transiently sufficient amount of lactate in the presence of BT may reverse the flow from
445 lactate to pyruvate in RI so that lactate is consumed instead of produced. In addition, under
446 the stress of an acidic pH BT may release toxins that contribute to the loss of viability of RI[49].
447 Some viable cells of RI may also grow by necrophagy on accumulated dead cells[50].
448
449 We found that interaction strength significantly varies in value and sign along the growth
450 curve, both with and without mucin (Figure 5), due to the variability in pH and nutrient

451 levels. Widely used population models such as the generalized Lotka-Volterra assume
452 interaction strengths to be constant. This is problematic, because even if environmental
453 conditions are controlled, bacteria themselves will frequently alter their environment and
454 hence their interaction mechanisms. Thus, the generalized Lotka-Volterra is only applicable
455 in systems where environmental conditions (including pH and nutrient levels) are kept
456 constant, i.e., chemostats. However, the intestine is not well described by a chemostat,
457 since nutrient levels, moisture and pH fluctuate with the daily intake of meals and the
458 circadian rhythm[51,52]. To cope with this, models can either account for changing interaction
459 strengths as done here, which requires knowledge of interaction mechanisms and
460 parameter values, or average over interaction strengths encountered in typical scenarios in
461 the environment of interest. Such an ensemble approach may better represent the
462 interaction between two organisms than the usual 'snapshot' strategy of quantifying
463 interactions in one particular condition but may not lend itself to predictive modelling. Thus,
464 changing interaction strengths are a challenge for predictive models of human gut
465 microbiota.
466
467 A deeper understanding of alternative metabolic strategies and changing species
468 interactions in gut microbiota is necessary to rationally design next-generation probiotics
469 and to predict the outcome of fecal microbiota transplantation. By deciphering the
470 interaction mechanisms between two common gut bacteria across changing conditions, we
471 have taken another step towards this goal.

472 ## Methods

473 **Human gut bacterial strains**

474 Bacterial strains of *Bacteroides thetaiotaomicron* VPI-5482 (DSM 2079^T) and *Roseburia*
475 *intestinalis L1-82* (DSM 14610^T) were obtained from the Deutsche Sammlung von
476 Mikroorganismen und Zellkulturen (DSMZ, Germany). The strains were frozen in Wilkins-
477 Chalgren Anaerobe Broth (WC; Oxoid Ltd., Basingstoke, United Kingdom) plus 20% glycerol
478 and maintained at -80°C until use.

479

480 **Mucin bead preparation**

481 Mucin beads were generated based on a protocol previously described[53]. Briefly, a
482 polysaccharide gel was prepared that contained (in g/100ml) Gelrite gellan gum (2.5, Sigma),
483 xanthan gum (0.25, Sigma) and porcine mucin type II (5, Sigma). The dried powders were
484 suspended in 100 ml of 90°C pre-heated distilled water containing 0.2 g sodium citrate
485 (Sigma) and 350 µl 10 M NaOH. This polymer solution was mixed until dissolved and then
486 autoclaved for 20 min at 121°C. The bead generation was performed under sterile
487 conditions and was based on a two-phase dispersion process[54]. The autoclaved solution was
488 cooled down to 50°C on a heater and slowly poured with a distance of 40 cm into 45°C
489 sunflower oil while keeping the stirring speed at 300 rpm, to obtain a suspension of aqueous
490 gel droplets in the hydrophobic phase. Gel beads were formed when cooling the suspension
491 down to 15°C on ice. The oil was carefully washed off the beads and were subsequently
492 hardened by soaking in a 0.1 M $CaCl_2$ solution (adjusted to pH 5.2) for 20 min at room
493 temperature. Beads with a diameter of 500 µm were selected by wet sieving and stored at
494 4°C in aseptic conditions until use.

495

496 **Cultivation and sample collection**

497 Serum bottles (120 ml) containing 60 ml WC medium and around 8000 mucin beads were
498 used as a spatially structured system to grow BT and RI. Briefly, each bottle was first filled
499 up with 50 ml WC medium, and 3 g wet mass of 500 µm mucin beads in 10 ml WC were
500 pipetted into each bottle under sterile conditions. The bottles were transferred to the
501 anaerobic workstation (Don Whitley A35 with HEPA filter; 10% H_2, 10% CO_2, 80% N_2, 55%
502 humidity) to be deoxygenated for 120 h. Oxygen-free conditions in liquid and in the beads

503 were confirmed through the color change of redox indicator resazurin. The bottles were
504 closed with rubber stoppers and sealed with aluminum caps that were UV sterilized for 1 h
505 in a laminar flow chamber before use. For bottle experiments without mucin beads, bottles
506 were filled with 60 ml WC medium that was prepared in the same way as described above.
507
508 To prepare the inoculum, 100 µl of BT or RI from frozen stocks was first inoculated in a 10-
509 ml Wheaton® serum vial containing 5 ml WC medium as preculture. These serum vials were
510 prepared by adding autoclaved medium and were deoxygenated in the anaerobic
511 workstation for 24 h. The vials were closed with rubber stoppers and sealed with aluminum
512 caps before use. After 16 h, each species' preculture was diluted to an OD_{600} of 0.1
513 determined by a portable spectrometer (DR1900; Hach Lange GmbH, Germany) and used as
514 the inoculum. The serum bottles were inoculated with 1 ml of the diluted preculture and
515 were incubated at a constant stirring rate of 170 rpm at 37°C in a shaker (KS 4000 i; IKA,
516 Staufen, Germany). Cultivations of the monoculture and co-culture were followed for 120 h
517 after inoculation, with samples taken from both the liquid and beads every four hours for
518 the first 48 h and every 12 h afterwards. For further investigation of the BT and RI growth
519 and their interactions in the long term, an experiment was designed for the co-culture to
520 follow for 288 h with samples taken every 24 h since 120 h after inoculation. All experiments
521 were performed at least in three biological replicates and always had a negative control
522 bottle without inoculation but with sampling for each time, to verify its sterility.
523
524 For each time point, two milliliters of the fermentation broth containing the suspended
525 mucin beads were sampled to 2-ml Eppendorf tubes. The serum bottles were opened and
526 closed carefully each time to avoid contamination in the anaerobic workstation. During
527 sampling the beads settled downward to the bottom within seconds. The supernatant was
528 carefully pipetted into new 2-ml tubes in the anaerobic workstation, and subsequently was
529 used to measure pH (PH-922, ISFET, Japan) and OD_{600}, and to profile metabolites as well as
530 quantify bacterial cell counts by live/dead staining followed by flow cytometry.
531
532 **Spent media and pH preference experiments**
533 Several spent media assays were conducted in serum bottles to clarify how BT and RI
534 interact with each other, and how abiotic factors influenced their growth. First, we grew a

535 culture of BT in WC for 48 h without mucin beads and sterilized the spent medium through a
536 filtration with 0.2-µm sterile syringe filters (PES; VWR International). In addition, the filtered
537 medium was autoclaved and deoxygenated for 24 h in the anaerobic workstation. Four
538 conditions were prepared to cultivate BT in the spent medium: *i*) the original spent medium
539 without adjusting pH (pH 5) and without adding carbon source, *ii*) spent medium with pH
540 adjusted to 6.5, *iii*) spent medium with glucose and pyruvate added in the same
541 concentrations as in WC at pH 5 and *iv*) spent medium with added glucose and pyruvate
542 plus pH adjusted to 6.5. We grew BT in three biological replicates in the serum bottles for a
543 period of 48 h and sampled every 8 h for measuring pH, OD_{600}, metabolites and live/dead
544 cell counts. Second, we grew BT in WC plus mucin beads in different periods of 24 h, 48 h
545 and 72 h, as well as in WC without mucin beads for 48 h. The cell- and oxygen-free spent
546 media were prepared in the same way as described above, and the pH was adjusted to 6.5
547 before autoclaving.
548
549 We further adjusted the pH of WC medium to the specified values of 4.5, 5.0, 5.5 and 6.0 by
550 using 1 M HCl solution, and also included the original WC of pH 6.7 for testing in serum
551 bottles. The growth of BT was followed for 48 h, with samples taken at 0 h, 4 h, 8 h, 12 h, 24
552 h, 36 h and 48 h and measured as described above.
553
554 **Detachment experiment**
555 To estimate the rate at which bacterial cells detach from particles, we followed the
556 population change of planktonic cells in a fresh WC medium, into which mucin beads with
557 attached BT cells were added. We first grew BT culture in a serum bottle containing 60 ml
558 WC plus mucin beads, with a same inoculum size as described: 1 ml of OD_{600} 0.1. After 24 h
559 cultivation, 2 ml of the fermentation broth containing the suspended mucin beads were
560 sampled to each 2-ml tube. 0.5 ml of phosphate buffer (PBS, pH 7.3; Invitrogen, Thermo
561 Fisher Scientific, USA) was added to each tube after washing with 1 ml PBS. The beads in PBS
562 were transferred to a 12-well plate (Sarstedt, Germany), with 1.5 ml sterile WC medium in
563 each well. For comparison, we included wells of fresh WC medium and WC medium
564 supplemented with sterile mucin beads, both of which were inoculated with BT in a volume
565 of 25 µl of OD_{600} 0.1. The experiment was performed in four replicate wells for each group.
566 The plates were incubated at a constant stirring rate of 170 rpm at 37°C, with liquid samples

567 taken at 0 min, 30 min, 60 min, 90 min, 150 min and 240 min for live/dead cell staining
568 followed by flow cytometry.
569
570 **Mucin monomeric sugar assays**
571 The degradation products of mucin including Neu5Ac, *N*-acetylgalactosamine, *N*-
572 acetylglucosamine, fucose, mannose and galactose were tested for their effects on the
573 growth of BT and RI. The tests for each species were performed by using 24-well plates
574 (Sarstedt, Germany) filled with two types of media: the standard WC and WC without
575 carbon sources glucose and pyruvate. Each medium was split into eight aliquots, each of
576 which was supplemented with either one of those mucin sugars in 1 mM or a mixture of all
577 six sugars together in 1 mM or without any sugar added as the control. We first inoculated
578 0.5 ml preculture with OD_{600} 0.1 to 20 ml of each aliquot, and then transferred 1.2 ml to
579 each well in three replicates. The plates of BT or RI were incubated at a constant stirring rate
580 of 120 rpm at 37°C for 24 h or 48 h, respectively, with samples taken every 12 h for pH and
581 live/dead staining followed by flow cytometry measurements.
582
583 **Quantification of bacterial cell counts using flow cytometry**
584 DNA-based staining using SYBR Green I (SG; Invitrogen) in combination with propidium
585 iodide (PI; Invitrogen) was applied to quantify and differentiate bacterial cells with intact
586 and damaged cytoplasmic membranes following the protocols described before[55]. Briefly,
587 the stock staining solution contained per ml: 10 µl of SG (10,000 concentrate), 20 µl of 20
588 mM PI and 970 µl of the filtered dimethylsulfoxide. Cells of the fresh culture in liquid from
589 different time points were diluted in a filter-sterilized PBS buffer for the live/dead cell
590 staining. Dilutions in 1:10 and 1:200 were performed for the first two time points (0 h and 4
591 h) and the later time points (after 8 h), respectively. Under anoxic conditions, cell samples
592 were diluted in 1-ml 96-well plates (Eppendorf, Germany) and stained with 3 µl of the SG/PI
593 staining solution, and subsequently were incubated for 20 min in the dark at 37°C right
594 before flow cytometric measurements. To quantify the attached cell counts, the mucin
595 beads sampled from different time points were first washed with 1 ml filter-sterilized PBS in
596 2-ml Eppendorf tubes. After carefully removing the supernatant, we added another 0.5 ml
597 PBS to the tubes that later were sonicated in the water bath of an ultrasonic cleaner
598 (USC200TH, VWR International) for 5 min at 45 kHz. The fresh detached cells in PBS were

599 diluted 1:10 in 96-well plates for all time points and were used for the live/dead cell staining
600 as described above. To confirm method accuracy, we also used a green fluorescent protein
601 (GFP)-tagged BT containing the modified plasmid pWW3452 (CTAATG in the start codon of
602 GFP was replaced with CATATG), which can stably fluoresce when grown anaerobically with
603 subsequent exposure to oxygen for 60 min as described[56].
604
605 Flow cytometric counts of the fresh cells were obtained by using a benchtop CytoFLEX S flow
606 cytometer (Beckman Coulter, Brea, USA), equipped with the identical lasers and detectors
607 as described previously[57]. Cell events of each sample were recorded for 1 min at a sample
608 flow rate of 10 µl/min, using the CytExpert software (version 2.4.0.28). Threshold values of
609 3,000 and 2,000 were applied to the channels of forward scatter and side scatter,
610 respectively. These thresholds were selected based on our tests of BT and RI cells from
611 different growth phases in serum bottles containing WC medium without and with mucin
612 beads to exclude most of the background events and to obtain accurate cell counts. In
613 addition to the instrumental calibration with CytoFLEX Daily QC Fluorospheres, 0.5 µm and 1
614 µm green fluorescent beads (Thermo Fisher Scientific, USA) were applied to each batch of
615 experiments as internal standards, to monitor the instrument stability and to make
616 cytometric data from different batches more comparable.
617
618 **Flow cytometry data processing**
619 We quantified the absolute abundance of BT and RI cells by clustering populations of flow
620 cytometric events in a UMAP[58] space using an in-house developed pipeline. The detailed
621 protocol and scripts necessary to reproduce our cell population counts are available in:
622 bit.ly/3WNrslL. Briefly, raw flow cytometry data from all channels were normalized and
623 scaled. Since flow cytometry data values have approximately log-normal distributions and
624 contain negative values, we first transformed the data with the arcsinh function and then
625 scaled it to exhibit uncorrelated unit variances and means around zero. Next, we projected
626 the transformed data into a three-dimensional UMAP space using the Euclidean distance as
627 a metric, and parameters of 25 and 0.1, respectively, for the number of neighbors and
628 minimum distance. Using flow cytometry runs containing cells and runs containing only the
629 media (blank) we first classified the events into four categories: (1) “live” (events in the
630 UMAP space that are SG positive, PI negative, and not in the blanks); (2) “inviable” (events

631 that are not in the blanks, but PI positive) and; (3) “debris” (events that are PI and SG
632 negative, but not in the blank files); (4) “blank” (events that overlap with the blank files). PI
633 and SG positive events were determined based on a threshold signal in their respective
634 channels, which were empirically estimated from the distribution of all the data samples.
635 The overlap with blank events from blank control files was determined by drawing a circle
636 around the points in the blank files, whose radius is proportional to the density of blank
637 points found in its proximity and classifying the area spanned by this circle as blanks in a
638 combined UMAP projection of samples and blank files. Only the events classified as “live”
639 were selected for the next steps. We then used the monoculture samples of each respective
640 time point to classify cells in the co-culture samples. For this we first built a training space
641 consisting of a labeled UMAP projection of 5,000 random events selected from each
642 monoculture biological replicate. Next, we used supervised UMAP to reproject the
643 monoculture and co-culture live-cell events into the training space. We then used the *K*-
644 nearest neighbors vote classifier (with parameters: n_neighbors = 50, weights = distance,
645 and metric = Mahalanobis) to identify the species labels of the co-culture events based on
646 their proximity to the monoculture events in the supervised UMAP space.
647
648 **Contamination check and confirmation of abundance profiles with 16S**
649 Sanger sequencing of 16S rRNA genes was performed regularly to check each strain identity.
650 In addition, we selected four biological replicates for each time point from the co-cultivation
651 experiments of BT and RI in WC with and without mucin beads, to confirm the abundance
652 profile of BT and RI in co-culture with 16S rRNA gene amplicon sequencing (Figure S15).
653 Sequencing of Illumina MiSeq and downstream analysis was performed as described
654 previously[57].
655
656 **Metabolite profiling**
657 Liquid samples in 1 ml were collected and centrifuged for 20 min at 21,130 × *g* at 4°C
658 (Centrifuge 5424 R; Eppendorf, Hamburg, Germany). The supernatants were used for
659 chemical analyses by applying external standards for calibration and quality control. The
660 concentrations of trehalose, glucose, pyruvate, succinate, formate, acetate, lactate, *n*-
661 butyrate and Neu5Ac were determined by high-performance liquid chromatography (HPLC).
662 HPLC analysis was carried out on an Agilent 1200 series HPLC system (Agilent Technologies,

663 USA) equipped with a Bio-Rad Aminex HPX-87H column (Biorad, USA) and a refractive index
664 detector at 40°C. Samples in 20 µl were injected and eluted for 60 min using 5 mM H_2SO_4 as
665 the mobile phase at a flow rate of 0.6 ml/min.
666
667 Concentrations of mucin monomeric sugars galactose, fucose and mannose were relatively
668 quantified by using gas chromatography, carried out by the Metabolomics Core of KU
669 Leuven. The supernatant in 100 µl of each sample was diluted to 1 ml in 80% methanol, and
670 left overnight to allow the precipitation of lipids and proteins. After centrifugation, 100 µl of
671 the supernatant was transferred and evaporated to dryness on a speedvac. A 2%
672 methoxyamine solution was prepared by dissolving methoxyamine hydrochloride (Supelco,
673 ref. 226904-5g) in pyridine (Sigma-Aldrich, ref. 270970-1L). 25 µl of the methoxyamine
674 solution was added to each sample and left to react for 1.5 h at 37°C for methoximation of
675 the hexoses. Afterwards, 80 µl N,O-Bis(trimethylsilyl)trifluoroacetamide with 1%
676 trimethylchlorosilane (Supelco, ref. 15238) was added and left to react for 30 min at 60°C
677 for trimethylsilylation of the methoximated hexoses. One microliter of each methoximated
678 and trimethylsilylated sample was injected by 2:1 split injection by an Agilent Technologies
679 7693 autosampler (Agilent Technologies, Santa Clara; California) into an Agilent 7890A gas
680 chromatograph equipped with a fused silica capillary column (0.25 mm × 30 m) with a
681 chemically bonded 0.25 µm HP-5MS stationary phase (Agilent J&W GC columns, Folsom;
682 California, ref 19091S-433). The injector temperature was 250°C, and the septum purge
683 flowrate was 3 ml/min. The helium flowrate through the column was 0.9 ml/min. The
684 column temperature was held at 160°C for 23.5 min and then increased by 100°C/min to
685 300°C, with 1 min holding there. The column effluent then entered an Agilent 7000 triple
686 quadrupole mass spectrometer. Temperatures of the transfer line and electron impact ion
687 source were 250°C and 230°C, respectively. Ions were generated by a 70 eV electron beam.
688 Spectra were acquired in a selected ion monitoring mode, with fragment masses based on
689 the analyses of standards.
690
691 **RNA extraction and sequencing**
692 We included a total of 69 samples representing the different growth phases of BT and RI in
693 three biological replicates for RNA sequencing, which were selected from the following six
694 independent experiments: monoculture BT in WC (4 h, 12 h and 36 h), monoculture RI in

695 WC (4 h, 12 h and 48 h), coculture BT and RI in WC (12 h , 24 h and 36 h), monoculture BT in
696 WC plus mucin beads (4 h, 12 h, 24 h, 48 h and 120 h), monoculture RI in WC plus mucin
697 beads (16 h, 28 h, 40 h and 48 h), and coculture BT and RI in WC plus mucin beads (6 h, 12 h,
698 24 h, 48 h and 120 h).
699
700 Total RNA was extracted and purified from RNAprotect-treated (Qiagen, Hilden, Germany)
701 frozen liquid samples using the phenol-free RNeasy Plus Mini Kit (Qiagen) according to the
702 instructions of the manufacturer. The cell pellets from -80°C were first lysed by adding the
703 lysis buffer RLT from the kit with 10 µl/ml β-mercaptoethanol. The content of PowerBead
704 tube (glass, 0.1 mm; Qiagen) was further added to the lysate prior to using a bead beater
705 (TissueLyser II, Qiagen) for homogenizing the mixture for 5 min at a speed of 30 beats/sec.
706 Other steps were performed by following the instructions. The eluted RNA samples in
707 nuclease free water were stored at -80°C until use. RNA concentrations were determined
708 with a Nanodrop ND 1000 spectrophotometer (Thermo Fisher Scientific, USA) and a Qubit
709 2.0 fluorometer using the Qubit RNA High Sensitivity Assay Kit (Invitrogen). Samples with a
710 260/230 absorbance ratio below 0.5 were purified using the MinElute PCR Purification kit
711 (Qiagen) and re-measured afterwards, with all samples above 0.5 after purification. RNA
712 integrity and yield were evaluated using RNA Nano and Pico 6000 LapChips (Agilent
713 Technologies, Santa Clara, CA, USA) run on an Agilent 2100 Bioanalyzer (Agilent
714 Technologies).
715
716 Library preparation and sequencing of RNA-seq libraries were carried out by the Genomics
717 Core of KU Leuven. All samples were prepared using the NEBNext Ultra II Directional RNA
718 Library Prep Kit for Illumina (New England BioLabs, #E7760L), with an initial amount of 100
719 ng total RNA in 12 µl. Bacterial rRNA depletion was proceeded using the rRNA Depletion Kit
720 (Bacteria) (New England BioLabs, #E7850L), and the residual mRNA was quality controlled
721 and converted to the libraries that were sequenced on a NovaSeq 6000 (Illumina, San Diego,
722 CA, USA) on a v1.5 S4_300 kit in XP mode. The resulting data of paired-end reads in fastq file
723 format were obtained and demultiplexed before further analysis.
724
725 **RNA-seq data processing**

726 The analysis of the raw sequencing reads was performed as follows: low quality reads and
727 adapters were trimmed with fastp[59]. High quality RNA reads were mapped to their
728 respective reference transcripts with Salmon run in a selective alignment mode and with a
729 decoy-aware index constructed from each organism's genome[60]. The latest reference
730 genomes and transcripts for BT and RI were downloaded from the ensemble bacteria[61] and
731 BV-BRC databases[62], respectively:
732 GCA_000011065.ASM1106v1.dna.toplevel.fa (*B. thetaiotaomicron* VPI-5482),
733 GCA_000011065.ASM1106v1.cds.all.fa (*B. thetaiotaomicron* VPI-5482),
734 GCA_900537995.1_Roseburia_intestinalis_strain_L1-82_genomic.fna (*R. intestinalis* L1-82),
735 GCA_900537995.1_Roseburia_intestinalis_strain_L1-82_cds_from_genomic.fna (*R.*
736 *intestinalis* L1-82). Raw transcript counts were extracted from salmon's output and used for
737 differential gene expression analysis. COG categories, COG numbers and KO numbers were
738 extracted from NCBI COG and KEGG database[63,64], for BT. To obtain annotations for RI, we
739 mapped the respective transcripts to the eggNOG database v5.0 with the eggNOG-mapper
740 2.1.9[65,66].
741
742 **Statistics**
743 To measure gene expression levels, we used methods of transcripts per million and
744 DESeq2's median of ratios to normalize RNA read counts[33]. A strict cutoff of at least 1.5-fold
745 change and a Benjamini-Hochberg adjusted *P* value less than or equal to 0.01 were applied
746 for differential gene expression calculated by DESeq2. Other statistical analyses were
747 performed using R (version 4.2.0).
748
749 **Modelling**
750 Details of model definition and parameterization can be found in the Supplementary file 2.
751
752 **Data availability**
753 Raw amplicon sequencing data and RNA-seq data have been deposited to the Sequence
754 Read Archive database under accession numbers of SAMN32182740-827 and
755 SAMN32321133-55, respectively (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA911421/
756 and https://www.ncbi.nlm.nih.gov/bioproject/PRJNA914119/). Raw flow cytometry data

757 have been deposited to flowrepository.org, under Repository IDs of FR-FCM-Z6YM, FR-FCM-
758 Z6YN, FR-FCM-Z6YP, FR-FCM-Z6YQ, FR-FCM-Z6YR, FR-FCM-Z6YS, FR-FCM-Z6YT, FR-FCM-
759 Z6YU, FR-FCM-Z6YV and FR-FCM-Z6YX, and will be made available upon acceptance of this
760 work. The processed RNA-seq data, flow cytometry data and fermentation data are
761 available at: https://github.com/msysbio.
762

763 **Code availability**

764 Flow cytometry data processing: bit.ly/3WNrslL, MATLAB file for the kinetic model and RNA-
765 seq processing scripts: https://github.com/msysbio.
766

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934

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949

950 **Author contributions**

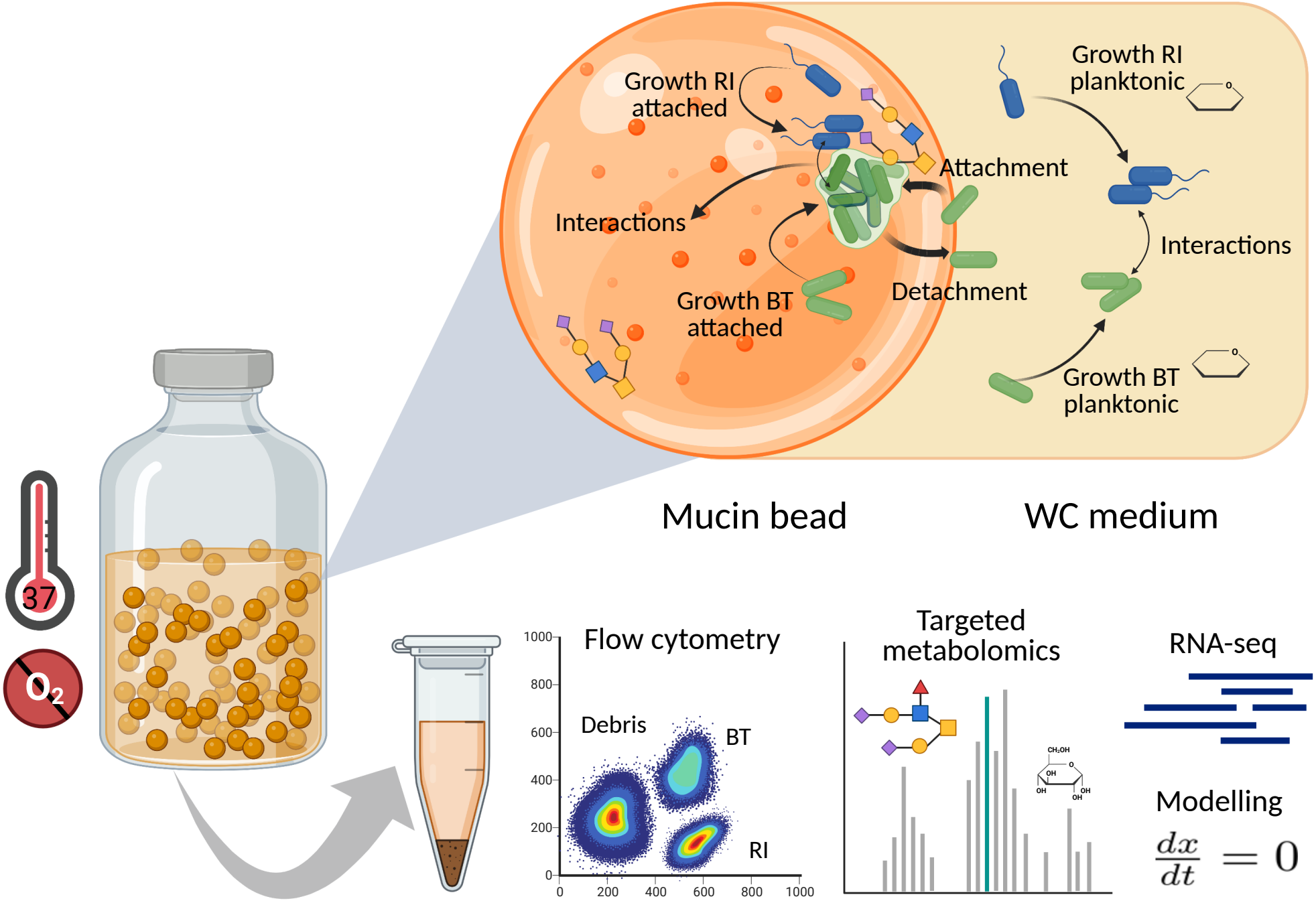
951 BL, DRG and KF designed the experiments and BL and DRG carried them out. AK processed
952 raw RNA-seq data. DG developed the kinetic model and performed simulations. KS
953 measured metabolite concentrations. AG and KB gave advice on experimental design and
954 interpretation. BL, DRG, KF and DG analyzed the results and BL, DRG and KF wrote the
955 manuscript. All authors read the manuscript.
956

957 **Competing interests**

958 None
959

960 **Additional information**

961 Supplementary file 1: supplementary figures.
962 Supplementary file 2: description of the kinetic model.
963 Supplementary file 3: RNA expression data for *Bacteroides thetaiotaomicron*.
964 Supplementary file 4: RNA expression data for *Roseburia intestinalis*.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Interactions
Growth BT attached
Attachment
Detachment
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Interactions
Growth BT planktonic
Mucin bead
WC medium
37
O_2
Flow cytometry
Debris
BT
RI
1000
800
600
400
200
0
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400
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Targeted metabolomics
RNA-seq
Modelling
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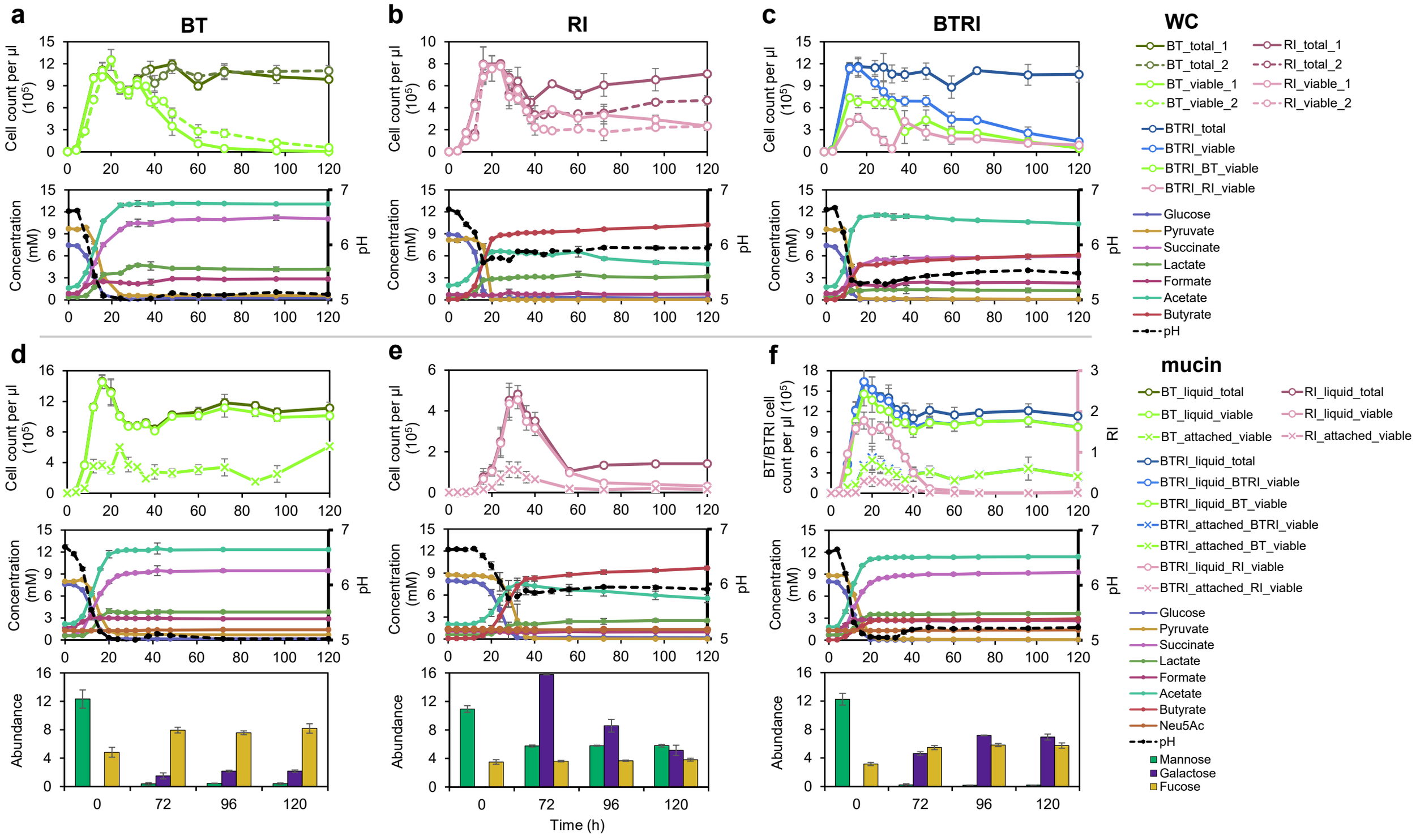
a
BT
b
RI
c
BTRI
WC
Cell count per µl (10^5)
Concentration (mM)
pH
BT_total_1
BT_total_2
BT_viable_1
BT_viable_2
RI_total_1
RI_total_2
RI_viable_1
RI_viable_2
BTRI_total
BTRI_viable
BTRI_BT_viable
BTRI_RI_viable
Glucose
Pyruvate
Succinate
Lactate
Formate
Acetate
Butyrate
pH
d
e
f
mucin
BT/BTRI cell count per µl (10^5)
RI
Abundance
Time (h)
BT_liquid_total
BT_liquid_viable
BT_attached_viable
RI_liquid_total
RI_liquid_viable
RI_attached_viable
BTRI_liquid_total
BTRI_liquid_BTRI_viable
BTRI_liquid_BT_viable
BTRI_attached_BTRI_viable
BTRI_attached_BT_viable
BTRI_liquid_RI_viable
BTRI_attached_RI_viable
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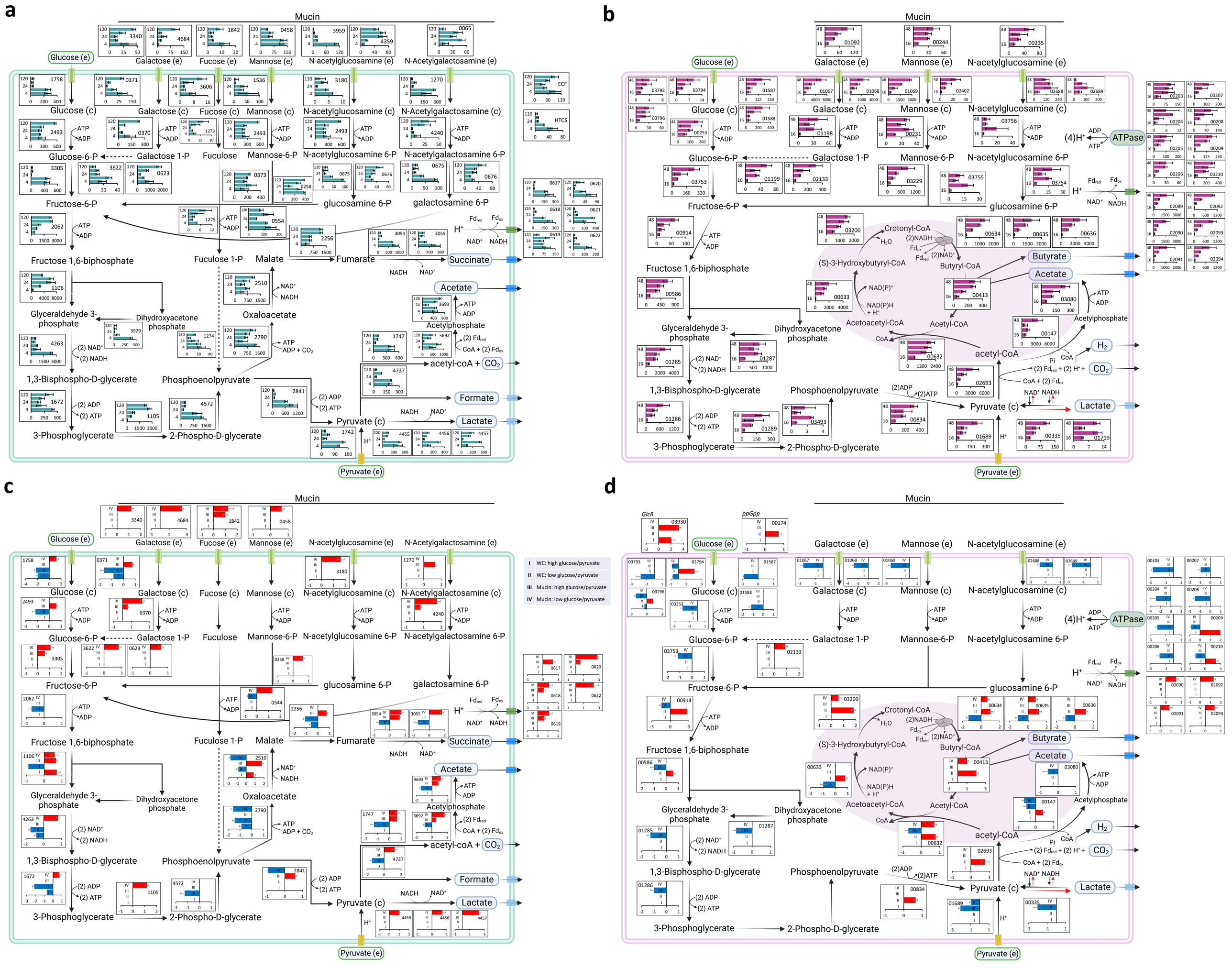
Butyrate
Neu5Ac
pH
Mannose
Galactose
Fucose


a
b
c
d
Mucin
Glucose (e)
Galactose (e)
Fucose (e)
Mannose (e)
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Glucose (c)
Galactose (c)
Fucose (c)
Mannose (c)
N-acetylglucosamine (c)
N-Acetylgalactosamine (c)
Glucose-6-P
Galactose 1-P
Fuculose
Mannose-6-P
N-acetylglucosamine 6-P
N-acetylgalactosamine 6-P
Fructose-6-P
glucosamine 6-P
galactosamine 6-P
Fructose 1,6-biphosphate
Fuculose 1-P
Malate
Fumarate
Succinate
Acetate
Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
Oxaloacetate
Acetylphosphate
acetyl-coA + CO2
1,3-Bisphospho-D-glycerate
Phosphoenolpyruvate
Formate
Pyruvate (c)
Lactate
3-Phosphoglycerate
2-Phospho-D-glycerate
Pyruvate (e)
ATP
ADP
ECF
HTCS
Crotonyl-CoA
(S)-3-Hydroxybutyryl-CoA
Butyryl-CoA
Butyrate
Acetoacetyl-CoA
Acetyl-CoA
Acetylphosphate
H2
CO2
(4)H+
ATPase
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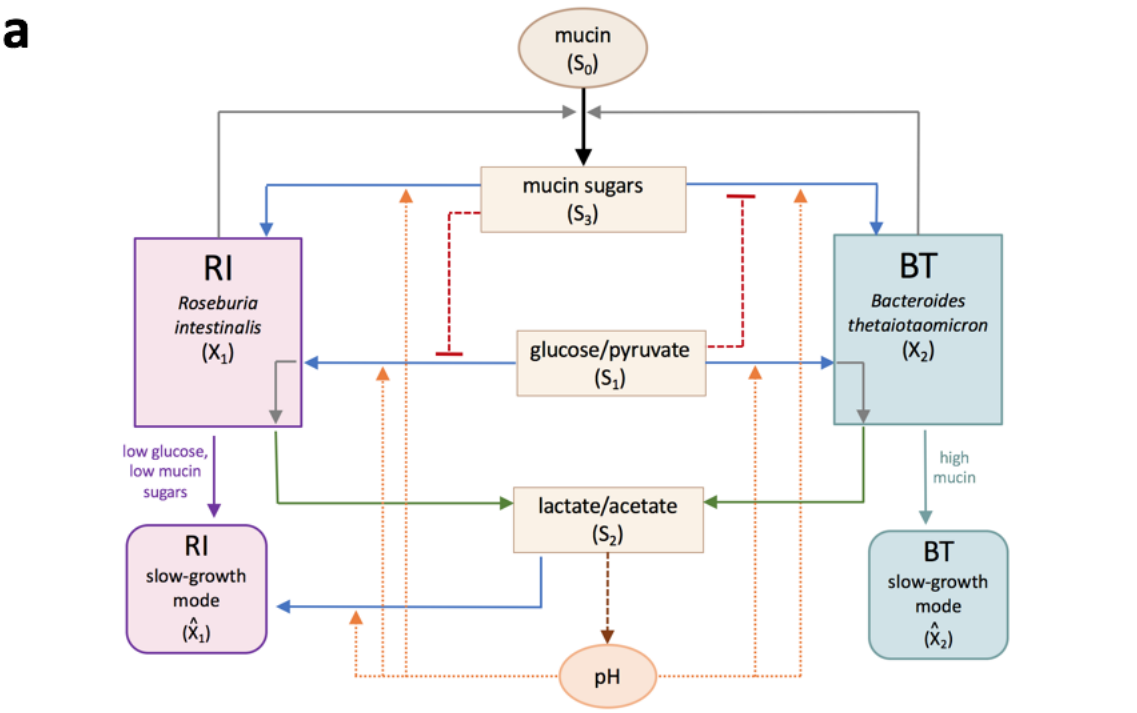
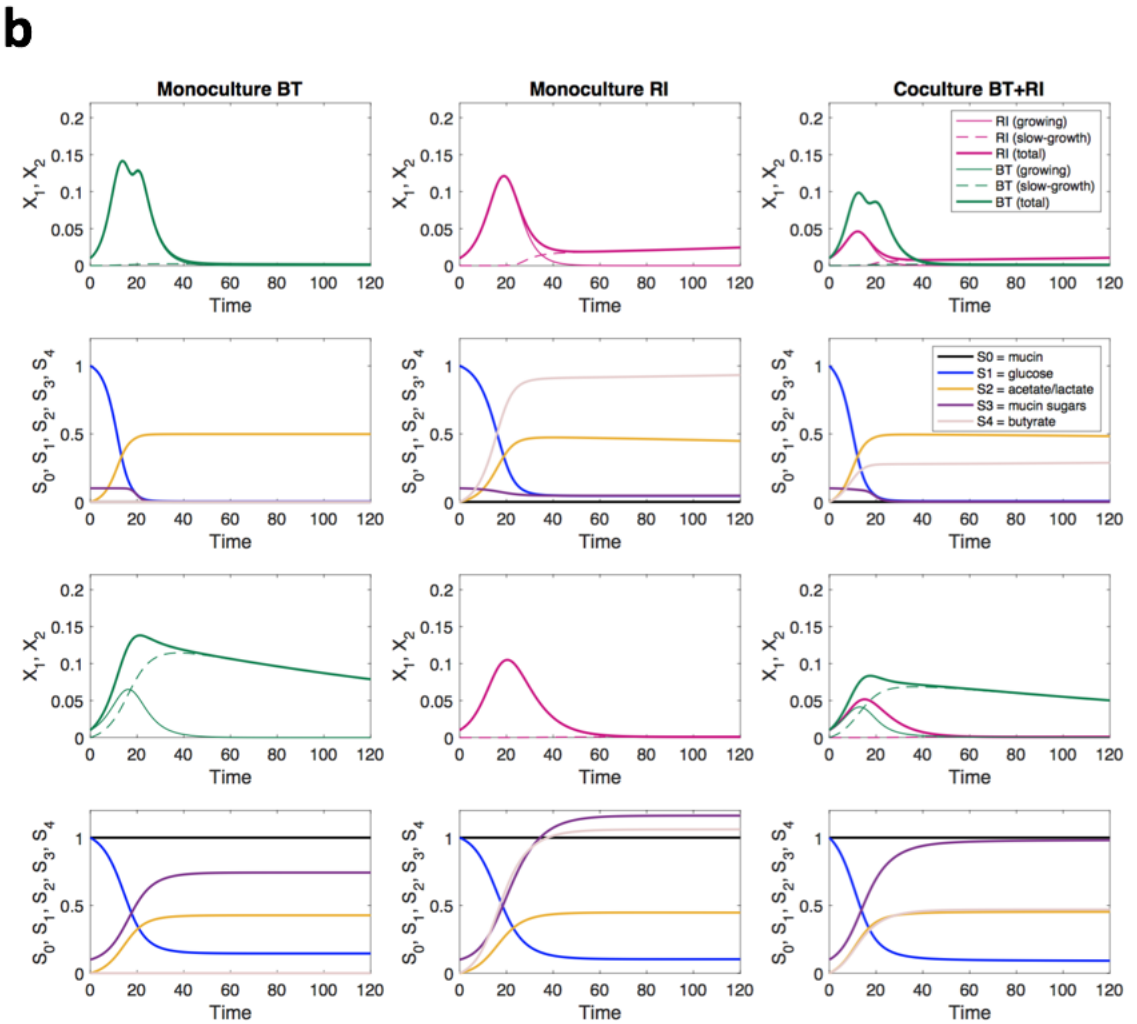
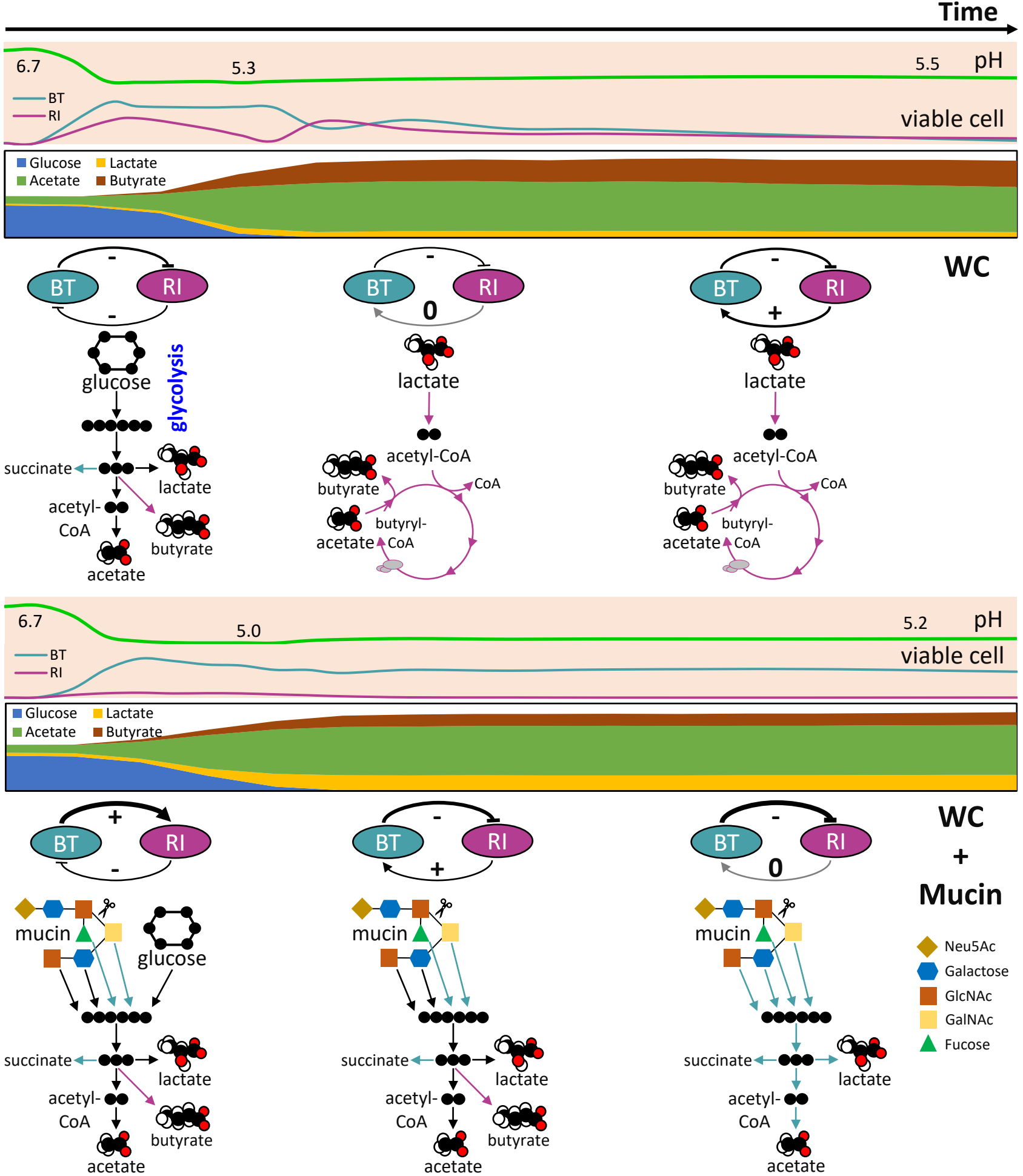
II WC: low glucose/pyruvate
III Mucin: high glucose/pyruvate
IV Mucin: low glucose/pyruvate
GlcR
ppGpp


a
mucin
(S_0)
mucin sugars
(S_3)
RI
Roseburia intestinalis
(X_1)
BT
Bacteroides thetaiotaomicron
(X_2)
glucose/pyruvate
(S_1)
low glucose, low mucin sugars
high mucin
lactate/acetate
(S_2)
RI
slow-growth mode
($\hat{X}_1$)
BT
slow-growth mode
($\hat{X}_2$)
pH


b
Monoculture BT
Monoculture RI
Coculture BT+RI
X_1, X_2
S_0, S_1, S_2, S_3, S_4
Time
RI (growing)
RI (slow-growth)
RI (total)
BT (growing)
BT (slow-growth)
BT (total)
S0 = mucin
S1 = glucose
S2 = acetate/lactate
S3 = mucin sugars
S4 = butyrate


Time
6.7
5.3
5.5
pH
BT
RI
viable cell
Glucose
Lactate
Acetate
Butyrate
WC
BT
RI
glucose
glycolysis
succinate
lactate
acetyl-CoA
butyrate
acetate
0
acetyl-CoA
CoA
butyrate
butyryl-CoA
acetate
+
6.7
5.0
5.2
pH
viable cell
WC
+
Mucin
mucin
glucose
Neu5Ac
Galactose
GlcNAc
GalNAc
Fucose