

1 **Exploring the interaction network of a synthetic gut bacterial** 2 **community**

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23 **Key Words**

24 Metabolic network, minimal consortium, syncom, cross-feeding, microbial community
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26

27 **Abstract**

28 A key challenge in microbiome research is to predict functionality from microbial community
29 composition. As central microbiota functions are determined by bacterial community networks
30 it is important to gain insight into the principles that govern bacteria-bacteria interactions. Here,
31 we focused on growth and metabolic interactions of the Oligo-Mouse-Microbiota (OMM¹²)
32 synthetic bacterial community, which is increasingly used as model system in gut microbiome
33 research. Using a bottom-up approach, we uncovered the directionality of strain-strain
34 interactions in mono- and pairwise co-culture experiments, as well as in community batch
35 culture. Metabolomics analysis of spent culture supernatant of individual strains in
36 combination with genome-informed pathway reconstruction provided insights into the
37 metabolic potential of the individual community members. Thereby, we could show that the
38 OMM¹² interaction network is shaped by both, exploitative and interference competition *in*
39 *vitro*. In particular, *Enterococcus faecalis* KBI was identified as important driver of
40 community composition by affecting the abundance of several other consortium members.
41 Together, this study gives fundamental insight into key drivers and mechanistic basis of the
42 OMM¹² interaction network, which serves as knowledge base for future mechanistic studies.

43

44 **Introduction**

45

46 The mammalian gastrointestinal tract harbors hundreds of bacterial species that occupy distinct
47 ecological niches (1, 2). Diversity and stable coexistence of community members after initial
48 assembly result in exclusion of invaders (3, 4). Community assembly and stability are
49 inherently driven by commensal or cooperative trophic interactions, in which metabolic by- or
50 end products of one species are the resource for another one (5-7). At the same time, bacteria
51 compete for substrates by employing diverse predatory mechanisms, like the production of
52 bacteriocins (8). These interaction patterns form complex ecological networks and determine
53 community-level functions of the microbiota including dietary breakdown, metabolite
54 production and colonization resistance (9-11). Consequently, disruption of bacterial networks
55 by antibiotics, disease or diet-mediated interventions results in impairment of community-level
56 functions (12, 13). To be able to predict, preserve and manipulate microbial community
57 function, it is important to identify functionally important members and understand relevant
58 interaction mechanisms between individual bacteria.

59 A multitude of different approaches have been used to characterize ecological networks of
60 microbial communities. Function-related patterns in native microbial communities can be
61 identified by systems biology approaches, combining metagenomics, metatranscriptomics and
62 metabolomics analyses (14). Together with stable-isotope probing methodologies
63 microorganisms with specific metabolic properties can be identified (15). Potentially
64 interacting species may be predicted from co-occurrence analysis supported by genome guided

65 metabolic modeling (16-18). To experimentally verify the key ecological, structural and
66 functional role of certain species in community structure and function, synthetic microbial
67 consortia provide several advantages over native communities. As they are well-characterized,
68 scalable and experimentally tractable, these systems are increasingly used to gain a mechanistic
69 understanding of gut microbial ecology (19-22).

70 The Oligo-Mouse-Microbiota (OMM¹²) is a synthetic bacterial community, which stably
71 colonizes mice and provides colonization resistance against enteropathogen infection (23-26).
72 The OMM¹² comprises twelve bacterial species (*Enterococcus faecalis* KB1,
73 *Limosilactobacillus reuteri* I49, *Bifidobacterium animalis* YL2, *Clostridium innocuum* I46,
74 *Blautia coccoides* YL58, *Enterocloster clostridioformis* YL32, *Flavonifractor plautii* YL31,
75 *Acutalibacter muris* KB18, *Bacteroides caecimuris* I48, *Muribaculum intestinale* YL27,
76 *Akkermansia muciniphila* YL44 and *Turicimonas muris* YL45), representing the five major
77 eubacterial phyla in the murine gastrointestinal tract (27) (**Fig. 1A**). The model is freely
78 available for non-commercial use (28), and is therefore increasingly employed in preclinical
79 microbiome research (29-32). So far little is known about the system's ecology and metabolic
80 capabilities, both of which are factors that determine assembly, population dynamics and
81 bacterial community functionality. Therefore, we aimed for a comprehensive exploration of
82 the metabolic potential (i.e., substrates, metabolism and end products) and interactions between
83 individual members of the OMM¹² consortium. We employed a bottom-up approach
84 connecting outcomes of mono- and pairwise co-culture experiments with observations from
85 complex communities in *in vitro* batch culture. Furthermore, we combined metabolomics
86 analysis of spent culture supernatants with genome-informed pathway reconstruction and
87 generated draft metabolic models of the OMM¹² consortium. Overall, we find that the majority
88 of *in vitro* strain-strain interactions is amensalistic or competitive. In accordance, bacteriocin
89 production and substrate overlap between the individual strains was correlated with negative
90 strain-strain interaction *in vitro*, revealing potentially underlying mechanisms. Together, this
91 work identified key interaction patterns among OMM¹² strains relevant in community
92 assembly and functionality.

93

94 **Results**

95 **Probing directional interactions of OMM¹² strains using spent culture media**

96 To characterize directional interactions of the OMM¹² consortium members, we chose an *in*
97 *vitro* approach to explore how the bacterial strains alter their chemical environment by growth
98 to late stationary phase.

99 Growth of the individual monocultures in a rich culture medium (AF medium, Methods, **Tab.**
100 **S1, Tab. S2**) was monitored over time (**Fig. S1**) and growth rates (**Tab. S3**) were determined.
101 Strains were grouped by growth rate (GR) into fast growing strains (GR > 1.5 h⁻¹, *E. faecalis*
102 KB1, *B. animalis* YL2, *C. innocuum* I46 and *B. coccoides* YL58), strains with intermediate
103 growth rate (GR > 1 h⁻¹, *M. intestinale* YL27, *F. plautii* YL31, *E. clostridioformis* YL32, *B.*
104 *caecimuris* I48 and *L.reuteri* I49) and slow growing strains (GR < 1 h⁻¹, *A. muris* KB18, *A.*
105 *muciniphila* YL44 and *T. muris* YL45). All strains reached late stationary phase within 20 h of
106 growth. To probe overlap in substrate requirements and interactions between the individual
107 OMM¹² members mediated by waste products or bacteriocins, sterile spent culture medium
108 (SM) after growth to late stationary phase of all strains was obtained. Each OMM¹² strain was

109 cultured in the SM of the other community members and their own SM and growth rate, the
110 area under the growth curve (AUC) and the pH were determined (**Fig. 1B, Fig. S2**).

111 A normalized inhibition factor (d_{AUC}) was determined by the AUC in SM relative to the AUC
112 in fresh AF medium ($d_{AUC} = \frac{AUC_{SM} - AUC_{AF}}{AUC_{AF}}$) to quantify the influence of the different SM on the
113 growth of the individual OMM¹² strains (**Fig. 1C**). Ten of the twelve SM were found to strongly
114 decrease ($d_{AUC} < -0.5$) the growth of at least one other strain of the consortium. Only the SM
115 of strains *A. muris* KB18 and *A. muciniphila* YL44 were found to strongly inhibit growth of
116 just the strains themselves. Corresponding to decreased AUC values in SM, growth rates were
117 found to be lower as well, resulting in linear correlation of AUC and growth rates (**Fig. S3, R**
118 > 0.5 , $p < 0.05$ for all strains). The SM of four strains, *E. faecalis* KB1, *B. coccoides* YL58, *E.*
119 *clostridioformis* YL32 and *B. caecimuris* I48, were found to strongly inhibit ($d_{AUC} < -0.5$) the
120 growth of nine other strains each (**Fig. 1C**). Notably, growth of *E. faecalis* KB1 itself was only
121 strongly reduced in its own SM, while it was able to grow in other strains' SM. *T. muris* YL45
122 was the only strain not showing clear growth inhibition in any of the SM while its SM strongly
123 decreased growth ($d_{AUC} < -0.5$) of three other strains, *A. muris* KB18, *M. intestinale* YL27 and
124 *F. plautii* YL31.

125 **Individual pH profiles as indicators for niche modification**

126 The pH of the culture medium after growth to stationary phase can be used as a measure for
127 the extent of strain specific environmental modification (11) and may partly explain inhibition
128 of bacterial growth in a SM. Therefore, we determined the pH of the individual SM before and
129 after (double spent media; DSM) growth of all OMM¹² strains (**Fig. 1B, Methods**). From these
130 values, we defined the ΔpH for every strain after growth in fresh medium (ΔpH_{SM}) and in all
131 SM (ΔpH_{DSM}) by analyzing the strength (difference of pH values) and direction (more acidic
132 or more alkaline) of the pH change (**Fig. 1D**). After growth in fresh AF medium with neutral
133 pH of 7.0, the OMM¹² strains showed different degrees of ΔpH_{SM} . While *E. faecalis* KB1, *B.*
134 *animalis* YL2, *M. intestinale* YL27, *B. caecimuris* I48 and *B. coccoides* YL58 distinctly
135 acidified the medium ($pH_{SM} < 6.2$), the growth of the other strains resulted in either slightly
136 more alkaline or nearly neutral medium. Correlating inhibition of growth in a SM (d_{AUC}) with
137 the mean pH of the individual SM for each strain revealed that growth inhibition did not directly
138 correlate with the pH. Only strains *B. animalis* YL2, *A. muciniphila* YL44 and *B. caecimuris*
139 I48 showed a significant negative correlation ($R < -0.5$, $p < 0.05$) between growth inhibition
140 and pH (**Fig. S4**) with stronger inhibition in more acidic pH ranges.

141 Most interestingly, many strains did not show the same magnitude or direction of alteration in
142 pH when grown in SM of another strain (ΔpH_{DSM}) compared to growth in fresh culture medium
143 (ΔpH_{SM}). This indicates an altered metabolic behavior of some strains in specific SM
144 environments that differs from metabolic behavior in fresh AF medium (**Fig. S5, Supplemental**
145 **Text A**).

146 **Production of antibacterial compounds by *E. faecalis* KB1**

147 Growth inhibition in SM (**Fig. 1C**) can further be explained by the production of antimicrobial
148 compounds. To test the production of antimicrobial compounds by the OMM¹² strains, we used
149 a phenotyping approach and performed spot assays on agar plates (**Fig. 1E**). Inhibition zones
150 were only seen in case of *E. faecalis* KB1, which produced one or several compounds active
151 against *B. animalis* YL2, *F. plautii* YL31, *E. clostridioformis* YL32, *C. innocuum* I46 and *L.*
152 *reuteri* I49. Genome analysis revealed that the strain encodes genes for the production of
153 several bacteriocins (Supplemental Text B), including enterocin L50, an enterococcal
154 leaderless bacteriocin with broad target range among Gram-positive bacteria (33). All other
155 strain pairs did not show signs of growth inhibition by compound excretion under these

156 conditions, despite the presence of genes for lanthibiotic production in the genome of *B.*
157 *coccoides* YL58 (determined by antiSMASH) (34). Although expression of antimicrobial
158 molecules may be induced by specific environmental triggers which are absent in the
159 monoculture *in vitro* setting, we concluded that interference competition may only play a role
160 in a subset of pair-wise interactions in AF medium involving *E. faecalis* KB1.

161 **Substrate depletion profiles correlate with growth inhibition in SM**

162 As pH and antimicrobial compounds only partly explained inhibition of growth in SM, we set
163 out to gain more insights into the individual metabolic profiles in our *in vitro* setting. Therefore,
164 triplicate samples of fresh AF medium and SM were analyzed by a mass spectrometry-based
165 untargeted metabolomics approach (TripleTOF, Methods). Combining positive and negative
166 ionization mode, 3092 metabolomic features were detected in total (Methods). From these,
167 2387 (77.20 %) were significantly altered (t-test, p value < 0.05) by at least one of the twelve
168 strains (**Fig. S6**). Hierarchical clustering of the metabolomic feature depletion profiles (i.e.
169 substrates used by the bacteria; **Fig. 2A**) reflects the phylogenetic relationship between the
170 strains (**Fig. 1A**). Correlating the phylogenetic distance between the individual strains with the
171 number of shared depleted metabolomic features in AF medium (**Fig. S7**) showed that
172 phylogenetically similar strains of the consortium have a higher substrate overlap than
173 phylogenetically distant strains ($R = -0.29$, $p = 0.017$). The total number of metabolomic
174 features that are depleted from AF medium greatly varies for the different strains, ranging from
175 over 600 depleted features for *M. intestinale* YL27 to only 42 for *A. muciniphila* YL44 (**Fig.**
176 **2B**). The strain specific profiles of depleted metabolomic features were compared pairwise and
177 the number of overlapping features was determined (**Fig. 2C**). Phylogenetically related strains
178 like *E. clostridioformis* YL32 and *B. coccoides* YL58 or *M. intestinale* YL27 and *B. caecimuris*
179 I48 share over 50% of depleted metabolic features each, suggesting a strong substrate overlap
180 in AF medium. Visualizing the extend of overlap between substrate depletion profiles reveals
181 that Bacteroidales, Clostridia and Bacilli strains of the consortium dominate with the highest
182 number of commonly depleted substrates in AF medium (**Fig. 2D**).

183 Correlating the growth inhibition in SM (d_{AUC}) with the pairwise overlap in depletion profiles
184 (**Fig. 2C**) revealed that a larger overlap is correlated with a stronger growth inhibition in the
185 corresponding SM ($R = -0.46$, $p = 3.1E-08$, **Fig. S8**). This is illustrated by *A. muciniphila*
186 YL44, which used only a low numbers of substrates from the AF medium (**Fig. 2B**) and the
187 SM of which had only little effect on the growth of the other strains of the consortium (**Fig.**
188 **1C**). On the other hand, the strain's growth itself was strongly reduced in the SM of most other
189 consortium members (**Fig. 1C**, **Fig. S2**), which depleted a large spectrum of metabolomic
190 features including those used by *A. muciniphila* YL44 (**Fig. 2C**).

191 **Genome-informed metabolic potential of the OMM¹² consortium**

192 To be able to infer metabolic interactions between the individual consortium members, a
193 reference dataset giving insight into the metabolic potential of the OMM¹² based on genetic
194 information was generated. We screened the genomes of the twelve strains for key enzymes of
195 central carbon metabolism (e.g., fermentation pathways, respiration and amino acid
196 metabolism) (**Fig. 3A**), as well as for transporters (ABC-transporters and PTS-systems) for
197 carbohydrates and amino acids (**Fig. S9, SI data table**). Hierarchical clustering of the genome-
198 informed metabolic potential (**Fig. 3A**) reflected phylogenetic relationships in several
199 instances, e.g., the Lachnospirales strains of the consortium *E. clostridioformis* YL32 and *B.*
200 *coccoides* YL58, as well as the Oscillospirales strains *F. plautii* YL31 and *A. muris* KB18 were
201 found to cluster closely together. Of note, the metabolic potential of *T. muris* YL45
202 (Sutterellaceae) was very distinct, clustering differently from all other strains. Generally, high
203 diversity of central and fermentation pathways was found among the consortium members.

204 Moreover, enzymes for the degradation of monosaccharides (e.g., arabinose, xylose and ribose)
205 and amino acids (e.g., methionine and glutamine) are highly prevalent among consortium
206 members. Phosphotransferase systems were especially prevalent among strains *E. faecalis*
207 KB1, *E. clostridioformis* YL32 and *C. innocuum* I46, while ABC transporters for
208 carbohydrates and amino acids were more distributed among all consortium members (**Fig.**
209 **S9**).

210

211 **Metabolite production and fermentation pathways of the OMM¹² strains in AF medium**

212 To gain insights into the metabolites and fermentation products produced and consumed by the
213 individual strains of the consortium in the given *in vitro* conditions, SM were analyzed using
214 different mass spectrometry approaches (Methods, **Fig. S10**, **Fig. S11**). Combining
215 experimentally obtained insights with genome-based information on the presence of key
216 enzymes enabled the generation of broad-scale draft metabolic models of the individual
217 OMM¹² community members (**Fig. 3B-E**, **Fig. S12**, Supplemental Text C).

218 To confirm that fermentation pathways identified by genomics were active under *in vitro*
219 conditions, short chain fatty acid (SCFA) production and consumption was analyzed (**Fig.**
220 **S10A**). As observed for the SM metabolic profiles (**Fig. S6**), hierarchical clustering revealed
221 that closely related bacteria showed similar SCFA production and consumption profiles. Both
222 Bacteroidetes strains produced acetic acid, succinic acid as well as branched-chain fatty acids.
223 Both Lachnospiraceae strains generated high amounts of acetic acid. Butyric acid is produced
224 by strains *F. plautii* YL31 and *C. innocuum* I46, the latter also being the only strain of the
225 consortium excreting valeric acid and hexanoic acid. Of note, *F. plautii* YL31 also consumed
226 lysine, indicating the ability to produce butyric acid from lysine, which was supported by the
227 presence of gene coding for lysine aminomutase (EC 5.4.3.2 and EC 5.4.3.3) as well as two of
228 the following genes encoding key enzymes in the pathway: L-erythro-3,5-diaminohexanoate
229 dehydrogenase (EC 1.4.1.11) and 3-keto-5-aminohexanoate cleavage enzyme (EC 2.3.1.247).

230 Formic acid was produced by several strains and consumed by *T. muris* YL45 and *B. coccooides*
231 YL58, indicating the ability of formic acid/ H₂ oxidation. *T. muris* YL45 and *B. coccooides*
232 YL58 both encode genes for a CO dehydrogenase/acetyl-CoA-synthase (EC 1.2.7.4 and EC
233 2.3.1.169), the key enzyme of the Wood-Ljungdahl pathway (reductive acetyl-CoA pathway).
234 Formic acid can be processed via this pathway to acetyl-CoA. As another prominent example
235 of bacterial fermentation, lactate production was confirmed (**Fig. S10B**) for *E. faecalis* KB1,
236 *B. animalis* YL2, *F. plautii* YL31, *A. muris* YL45, *C. innocuum* I46 and *B. caecimuris*, all of
237 which harbor genes coding for the enzyme lactate dehydrogenase (EC 1.1.1.27 and EC
238 1.1.1.28).

239 By quantifying amino acid levels we could show that Bacteroidetes and Lachnospiraceae
240 strains exhibited similar amino acid depletion and production profiles. In SM of strains *M.*
241 *intestinale* YL27 and *B. caecimuris* I48, elevated levels of a diverse range of amino acids
242 including glutamic acid, histidine, methionine, proline and phenylalanine were detected.
243 Lachnospiraceae strains showed increased levels of isoleucine, tryptophan and valine, while
244 alanine was especially depleted by *B. coccooides* YL58. Other strains of the consortium showed
245 specific depletion of single amino acids, e.g., *F. plautii* YL31 strongly depleted lysine and
246 glutamic acid, while *E. faecalis* KB1 depleted serine.

247 **Growth of OMM¹² strains in pairwise co-culture**

248 Next, we performed a set of experiments to characterize strain-strain interactions in the
249 dynamic community-dependent context. We first analyzed direct competition of all strains in
250 pair-wise co-cultures over the course of 72h, with serial dilutions every 24h. While growth was

251 monitored continuously by OD 600nm, samples for pH measurements and qPCR analysis were
252 taken every 24h. The growth curves of most co-cultures, as well as supernatant pH differed
253 from the corresponding strain specific characteristics observed in monoculture (**Fig. S13, Fig.**
254 **4A**). These differences reflect co-culture dynamics, as can be seen from change in relative
255 abundances over time (**Fig. 4B, C**).

256 To identify directionality and mode of interaction between the OMM¹² strains, we analyzed the
257 relative changes in absolute abundance (16S rRNA gene copies) as a measure of how
258 successful a strain can grow in co-culture relative to monoculture after 72h. The mean absolute
259 abundance ratio was calculated for every strain in all pairwise co-cultures ($r_{i,bm} = \frac{m_{i,co}(t72h)}{m_{i,mono}(t72h)}$)
260 (**Fig. S14, Methods**). If absolute abundance of a strain increased significantly in co-culture
261 relative to monoculture ($r_{bm} > 1$), the interaction was categorized as positive (+), if it decreased
262 ($r_{bm} < 1$) the interaction was categorized as negative (-) (t-test comparing the r_{bm} of three
263 independent experiments, **Fig. S15**). If it did not significantly ($p > 0.05$) differ from that in
264 monoculture ($r_{bm} = 1$), the interaction was categorized as neutral (0). By this, we created a co-
265 culture interaction matrix (**Fig. 5A**): the vast majority of the interactions was classified as
266 amensalistic (0/- and -/0, 46 of 66 of interactions). A smaller subset of interactions was either
267 competitive (-/-, 7 of 66 of interactions) or neutral (0/0, 11 of 66 of interactions). No mutualistic
268 interactions (+/+) were observed. However, one example for each, commensalism (0/+ and
269 +/0) and predation (+/- and -/+), were identified.

270 The extent to which the individual strains altered the growth of other community members in
271 the co-culture differed distinctly. While *E. faecalis* KB1 and *C. innocuum* I46 lead to nine
272 negative co-culture outcomes each, *A. muciniphila* YL44 and *A. muris* KB18 only impaired
273 growth of one and zero strains, respectively. Simultaneously, both strains are negatively
274 influenced in most co-cultures, with a significantly decreased absolute abundance in ten and
275 eight co-cultures, respectively. Notably, *B. coccoides* YL58 is involved in five of seven
276 competitive interactions of the consortium. These observations are in line with the outcomes
277 observed in SM experiments, as strongly negative co-culture outcome correspond to a strong
278 inhibition of a strain in the respective SM (**Fig. S16**).

279 **Community structure of the OMM¹² consortium**

280 Next, we set out to investigate if interactions found in co-cultures are transferrable to the
281 strains' behavior in the complete OMM¹² community. To this end, all twelve OMM strains
282 were simultaneously co-cultured in AF medium and were serially diluted 1:100 every 24h into
283 fresh AF medium. Relative abundance of all strains after 72h and 10 days compared to the
284 inoculum was determined by qPCR for ten replicates each in two independent experiments
285 from different inocula (**Fig. S17, Fig. S18, Fig. 5B**).

286 While each of the OMM¹² members except *E. faecalis* KB1 was outcompeted to a very low
287 relative abundance in at least one pairwise culture (**Fig. 5A**), the majority (10 out of 12) of the
288 consortium members were able to coexist in the complex community over the course of 72h
289 (**Fig. S17A**) and up to 10 days. (**Fig. 5B, Fig. S17B**). Replicate communities showed
290 reproducible community structure, even when different inocula were used (**Fig. 5B, Fig. S17B**)
291 and especially when compared on the order and phylum level (**Fig. S18**). Communities were
292 dominated by *B. coccoides* YL58 and *E. faecalis* KB1, together making up > 50% of the
293 relative abundance, which corresponds to their dominant role in SM and co-culture experiments
294 (**Fig. 1C, 5A**). While strains *B. animalis* YL2 and *L. reuteri* I49 were not detectable at 72h and
295 10 days in all replicates, *A. muris* KB18 was found in only few of the communities at 10 days
296 (relative abundance < 1%).

297 ***E. faecalis* KB1 strongly impacts overall community composition**

298 To understand how interactions observed on the pairwise level transfer to the community
299 context, we derived pairwise correlations from the relative abundance data of all communities
300 at day 10 derived from two independent experiments (**Fig. S19**). The strong negative influence
301 of *E. faecalis* KB1 on most other strains observed in pairwise co-cultures applied in the
302 community context as well. High relative abundance of *E. faecalis* KB1 linearly correlated
303 with decreased relative abundance of *C. innocuum* I46 ($R = -0.87$; $p < 0.05$), supporting the
304 hypothesis of a predatory interaction between *E. faecalis* KB1 and *C. innocuum* I46 as observed
305 in co-culture experiments (**Fig. 5A, D**). In order to identify potentially cross-fed metabolites of
306 *C. innocuum* I46 to *E. faecalis* KB1, we mined metabolomic data of SM for features enriched
307 in *C. innocuum* I46 and depleted by *E. faecalis* KB1. Thereby, we identified several compounds
308 including malate, L-methionine, spermidine and methylglyoxal (**Fig. 5E**). To experimentally
309 support the idea of cross-feeding, we exemplarily tested uptake of ^{14}C -malate into intact cells
310 of *E. faecalis* KB1. To slow the metabolization of this metabolite, all assays were performed
311 at 18°C . We found a very fast linear uptake of ^{14}C -malate by *E. faecalis* KB1 within the first
312 60 s, which could explain why malate utilization confers a growth advantage to this strain (**Fig.**
313 **5F**).

314 Finally, we were interested in how the absence of *E. faecalis* KB1 would affect the overall
315 community structure. We generated a ‘dropout’ community including all strains of the OMM¹²
316 consortium except *E. faecalis* KB1 (OMM¹¹-KB1). Compositional analysis revealed increased
317 relative abundance of *C. innocuum* I46 and *B. animalis* YL2 in the OMM¹¹-KB1 compared to
318 the full OMM¹² community (**Fig. 5C**). In addition, the absolute abundances of strains *B.*
319 *animalis* YL2, *C. innocuum* I46 and *B. caecimuris* I48 were found to increase significantly (t-
320 test, $p < 0.05$) in the absence of *E. faecalis* KB1 (**Fig. S20**). While the increase in abundance
321 of *B. animalis* YL2 and *C. innocuum* I46 may be explained by absent enterocin production by
322 *E. faecalis* KB1, the increased abundance of *B. caecimuris* I48 was unexpected. Further, the
323 abundance of *F. plautii* YL31, *E. clostridioformis* YL32, *A. muciniphila* YL44 and *T. muris*
324 YL45 was found to decrease in the absence of *E. faecalis* KB1. This indicates either direct
325 positive effects of *E. faecalis* KB1 on these strains or indirect effects that occur through the
326 overall shift in OMM¹¹-KB1 community composition compared to the OMM¹² consortium.

327

328 Discussion

329 A central challenge in gut microbiome research is to understand how interactions between the
330 individual microorganisms affect community-level structure and related functions. Bottom-up
331 approaches involving synthetic communities are valuable tools to study these interactions, as
332 they allow to reduce complexity and to enable strain-specific manipulation. Using an *in vitro*
333 approach, we focused on characteristics and interactions of the OMM¹² community and
334 combined monoculture, pairwise and community cultivation of the strains with genome and
335 metabolomics analysis of their SM. Thereby we reveal that the OMM¹² community interaction
336 network is shaped by exploitative and interference competition. In particular, *E. faecalis* KB1,
337 a low-abundant member of the mammalian gut microbiota, was identified as important driver
338 of *in vitro* community composition by directly or indirectly altering the abundance of several
339 other consortium members. We provide draft metabolic models of the individual OMM¹²
340 strains, which will be a valuable tool for mechanistic studies using this synthetic community.

341 Exploitative (i.e. substrate) competition plays a major role in shaping intestinal bacterial
342 communities (35). Understanding the underlying principles of how bacteria compete for

343 available nutrients is essential to predict and control community composition. We found that
344 phylogenetically similar strains showed a higher substrate overlap (**Fig. S7**), which is in
345 accordance with previous studies demonstrating that phylogeny reflects metabolic capabilities
346 of bacteria (36, 37). Furthermore, overlap in substrate depletion profiles was correlated with
347 growth inhibition in the respective SM (**Fig. S8**). This clearly indicates strong exploitative
348 competition between individual OMM¹² strains. In particular *B. caecimuris* I48, *E. faecalis*
349 KB1, *E. clostridioforme* YL32 and *B. coccoides* YL58 were found to consume a high number
350 of substrates (>200), while their SM inhibited growth of the majority of the other community
351 members (**Fig. 1C**). Of note, *M. intestinale* YL27 and *C. innocuum* I46, also consumed over
352 200 substrates each, but inhibited few other strains. This demonstrates that substrate overlap
353 cannot simply predict inhibition in all cases and other mechanisms (i.e. waste product
354 inhibition) play a role in specific cases.

355 Besides substrate competition, a strain's ability to acidify its environment or release an
356 inhibitory factor (e.g. waste products, bacteriocins) can determine if another species can grow
357 in the exhausted medium or not. Several strains, including *B. caecimuris* I48, *B. coccoides*
358 YL58, *E. faecalis* KB1 and *C. innocuum* I46 acidified the medium during growth in
359 monoculture. However, only for few species, *A. muciniphila* YL44, *B. caecimuris* I48 and *B.*
360 *animalis* YL2, acidic pH correlated with reduced growth (**Fig. S4**). Moreover, the pH in the
361 full OMM¹² community, where most of the strains coexisted, was also acidic (pH of 6.2),
362 suggesting that pH modification does not play a major role in driving community composition
363 *in vitro*. Interference competition by bacteriocins is widespread among gut bacterial
364 communities (38). We found that *E. faecalis* KB1 produces at least one antimicrobial
365 compound that shows activity against five of the Gram-positive OMM¹² strains (*B. animalis*
366 YL2, *E. clostridioformis* YL32, *F. plautii* YL31, *C. innocuum* I46 and *L. reuterii* I49) (**Fig.**
367 **1E**). *E. faecalis* harbors genes coding for at least two different enterocins (enterocin
368 L50A/L50B and enterocin O16). Therefore, we hypothesize that some of the inhibitory effects
369 of *E. faecalis* KB1 on those strains can be attributed to enterocin-mediated killing.

370 *E. faecalis* is a prevalent but low abundant member of the undisturbed human and animal
371 microbiota. Following antibiotic therapy, the bacterium can dominate the gut and cause blood-
372 stream infection in immunocompromised individuals (39). Understanding how *E. faecalis* out-
373 competes/overgrows other gut microorganisms is important in order to intervene with *E.*
374 *faecalis* domination in the gut. Besides enterocin-mediated killing we found that metabolite
375 cross-feeding seems to contribute to the interaction of *E. faecalis* KB1 with *C. innocuum* I46
376 (**Fig. 5D; Fig. S10**). Based on metabolic profile mining, we hypothesize, that *E. faecalis* KB1
377 consumes malate, methionine, arginine and serine among other metabolites in co-culture with
378 *C. innocuum* I46 (**Fig. 5E, Fig. S11**). Interestingly, a previous study (40) reported that glucose-
379 malate co-metabolism increases growth of *E. faecalis* over glucose consumption alone. In
380 connection with fast uptake rate of ¹⁴C-malate by *E. faecalis* KB1 (**Fig. 5F**), this suggests that
381 malate cross-feeding may also contribute to *E. faecalis* KB1 gain in absolute abundance in co-
382 culture with *C. innocuum* I46.

383 Using batch culture experiments we were able to investigate assembly and dynamics of full
384 OMM¹² and OMM¹¹-KB1 dropout communities *in vitro*. A significant increase in *B. animalis*
385 YL2 and *C. innocuum* I46 in the community lacking *E. faecalis* KB1 suggested that enterocin-
386 mediated killing also shapes the more complex community (**Fig. S20**). Notably, in the full
387 OMM¹² community, ten of the twelve strains co-existed over ten days. This was remarkable
388 given the high number of negative pairwise interactions in SM and co-culture experiments.
389 Differences between the behavior of strains in pairwise versus complex communities point at
390 higher-order ecological interactions that emerge in the community context. As previously

391 shown in other studies, the underlying mechanisms may involve metabolic flexibility or mixed
392 substrate utilization of the strains in the presence of competitors (41), metabolite cross-feeding
393 and lack of waste-product inhibition and overall change in pH (11) or an excess of provided
394 substrates in the medium.

395 Following up, it will be important to assess, if the *in vitro* findings can be translated to the
396 mouse model. Several differences between *in vitro* and *in vivo* conditions were noted. While
397 the *in vitro* community is dominated by *B. coccoides* YL58 and *E. faecalis* KB1, mouse
398 communities are dominated by *B. caecimuris* I48 and *A. muciniphila* YL44 (**Fig. S21**)(26).
399 Enrichment of amino acids and glucose in the used culture medium may favor growth of *B.*
400 *coccoides* YL58 and *E. faecalis* KB1 *in vitro* at the expense of bacteria specialized on
401 utilization of mucin and other complex carbohydrates (42, 43). To this end, it will be
402 worthwhile to modify the *in vitro* conditions to more closely recapitulate the chemical
403 landscape and spatial structure of the gut in future experiments.

404 Concluding, our study presents a comprehensive *in vitro* investigation of strain-strain
405 interactions between members of a widely used synthetic intestinal bacterial community.
406 Characterization of the metabolic profile of individual strains of the consortium as well as
407 analyzing their metabolism and community assembly in co-culture revealed *E. faecalis* KB1
408 and *B. coccoides* YL58 to be important drivers of community composition. Drawing on this
409 detailed understanding of *in vitro* behavior, our results will enable to employ this model for
410 mechanistic *in vivo* studies. This step-wise approach may ultimately allow accurate description
411 of interaction dynamics of *in vivo* gut microbial communities and pave the way for targeted
412 manipulation of the microbiome to promote human health. In particular, extending the
413 approach of dropout communities lacking specific strains could help to elucidate the role of
414 individual players in community functions like dietary breakdown, metabolite production and
415 colonization resistance and to identify general principles of how bacterial interaction networks
416 and the corresponding emergence of higher order interactions shape microbiome function. This
417 will enable the design of therapeutic interventions to control microbial community functions
418 by advanced microbiome engineering.

419

420 **Methods**

421 **Generation of a 16S gene based phylogenetic tree**

422 The genomes of the twelve strains of the OMM¹² consortium (27) were accessed via
423 DDBJ/ENA/GenBank using the following accession numbers: CP022712.1, NHMR02000001-
424 NHMR02000002, CP021422.1, CP021421.1, NHMQ01000001-NHMQ01000005,
425 NHTR01000001-NHTR01000016, CP021420.1, NHMP01000001-NHMP01000020,
426 CP022722.1, NHMU01000001-NHMU01000019, NHMT01000001-NHMT01000003,
427 CP022713.1 and annotated using Prokka (default settings) (44). The 16S rRNA sequences of
428 all strains were obtained. These rRNA FASTA sequences were uploaded to the SINA Aligner
429 v1.2.11 (45) to align these sequences with minimum 95% identity against the SILVA database.
430 By this, a phylogenetic tree based on RAxML (46), GTR Model and Gamma rate model for
431 likelihood was reconstructed. Sequences with less than 90% identity were rejected. The
432 obtained tree was rooted using *midpoint.root()* in the phytools package (47) in R and visualized
433 using iTOL online (48).

434

435 **Genome annotation for predicting genome-informed metabolic potential**

436 The genomes (accessed via DDBJ/ENA/GenBank as stated above) of the twelve strains of the
437 OMM¹² consortium were annotated using prodigal (version V2.6.3, default settings) (49) and
438 KEGG orthologies (KO) for the protein-coding genes were obtained using the tool KOfamscan
439 (default settings) (50). The tool provided multiple KO annotations for each gene with
440 corresponding e-values and threshold scores. In order to get one KO annotation per gene, the
441 annotation was considered only if a) the internal threshold score was reached (marked ‘*’ by
442 kofamscan) or b) an evalue of > 1e-03 was reached. The remaining annotations were ignored.

443 **Strains and culture conditions**

444 Bacterial cultures were prepared from frozen monoculture stocks in a 10ml culture and
445 subculture in cell culture flasks (flask T25, Sarstedt) previous to all experiments. Cultures were
446 incubated at 37°C without shaking under strictly anaerobic conditions (gas atmosphere 7% H₂,
447 10% CO₂, 83% N₂). All experiments were carried out using AF medium (18 g.l⁻¹ brain-heart
448 infusion, 15 g.l⁻¹ trypticase soy broth, 5 g.l⁻¹ yeast extract, 2.5 g.l⁻¹ K₂HPO₄, 1 mg.l⁻¹ haemin,
449 0.5 g.l⁻¹ D-glucose, 0.5 mg.l⁻¹ menadione, 3% heat-inactivated fetal calf serum, 0.25 g.l⁻¹
450 cysteine- HCl·H₂O). The following strains were used in this study: *Enterococcus faecalis* KB1
451 (DSM32036), *Bifidobacterium animalis* YL2 (DSM26074), *Acutalibacter muris* KB18
452 (DSM26090), *Muribaculum intestinale* YL27 (DSM28989), *Flavonifractor plautii* YL31
453 (DSM26117), *Enterocloster clostridioformis* YL32 (DSM26114), *Akkermansia muciniphila*
454 YL44 (DSM26127), *Turicimonas muris* YL45 (DSM26109), *Clostridium innocuum* I46
455 (DSM26113), *Bacteroides caecimuris* I48 (DSM26085), *Limosilactobacillus reuteri* I49
456 (DSM32035), *Blautia coccooides* YL58 (DSM26115).

457 **Growth measurements**

458 Bacterial growth was measured in 96well round bottom plates (Nunc) using a GenTech Epoch2
459 plate reader. Inocula were prepared from a previous culture and subculture and diluted in fresh
460 AF medium to 0.01 OD_{600nm}. Absorption at wavelength 600nm was determined in a reaction
461 volume of 100 µl in monoculture and SM experiments and 150 µl in co-culture experiments.
462 During continuous measurements, the plate was heated inside the reader to 37°C and a 30
463 second double orbital shaking step was performed prior to every measurement.

464 **Generation of spent culture media**

465 Bacterial cultures and subcultures were grown for 24 hours each in 10ml AF medium at 37°C
466 under anaerobic conditions without shaking. Bacterial spent culture supernatants (SM) were
467 generated by centrifugation of the densely grown subculture at 4°C for 20min at 5000xg and
468 subsequent pH measurement and filter-sterilization (0.22 µm). SM samples were aliquoted and
469 immediately frozen at -80°C. Samples were thawed under anaerobic conditions previous to
470 growth measurements. Growth of all bacterial monocultures in the spent culture media (SM)
471 of all respective other strains was then measured as described above. SM were inoculated with
472 bacterial monocultures with starting OD_{600nm} 0.01. After monoculture growth of 20h in the
473 respective SM (resulting in double spent media, DSM), pH values were determined.

474 **pH measurements**

475 pH measurements of bacterial supernatants were performed using a refillable, glass double
476 junction electrode (Orion™ PerpHecT™ ROSS™, Thermo Scientific).

477 **Metabolic profiling of late stationary phase bacterial supernatants**

478 The untargeted analysis was performed using a Nexera UHPLC system (Shimadzu) coupled to
479 a Q-TOF mass spectrometer (TripleTOF 6600, AB Sciex). Separation of the spent media was
480 performed using a UPLC BEH Amide 2.1x100, 1.7 µm analytic column (Waters Corp.) with
481 400 µL/min flow rate. The mobile phase was 5 mM ammonium acetate in water (eluent A) and

482 5 mM ammonium acetate in acetonitrile/water (95/5, v/v) (eluent B). The gradient profile was
483 100% B from 0 to 1.5 min, 60% B at 8 min and 20% B at 10 min to 11.5 min and 100% B at
484 12 to 15 min. A volume of 5 μ L per sample was injected. The autosampler was cooled to 10°C
485 and the column oven heated to 40°C. Every tenth run a quality control (QC) sample which was
486 pooled from all samples was injected. The spent media samples were measured in a randomized
487 order. The samples have been measured in Information Dependent Acquisition (IDA) mode.
488 MS settings in the positive mode were as follows: Gas 1 55, Gas 2 65, Curtain gas 35,
489 Temperature 500°C, Ion Spray Voltage 5500, declustering potential 80. The mass range of the
490 TOF MS and MS/MS scans were 50 - 2000 m/z and the collision energy was ramped from 15
491 - 55 V. MS settings in the negative mode were as follows: Gas 1 55, Gas 2 65, Cur 35,
492 Temperature 500°C, Ion Spray Voltage -4500, declustering potential -80. The mass range of
493 the TOF MS and MS/MS scans were 50 - 2000 m/z and the collision energy was ramped from
494 -15 - -55 V.

495 The “msconvert” from ProteoWizard (51) were used to convert raw files to mzXML (de-noised
496 by centroid peaks). The bioconductor/R package xcms (52) was used for data processing and
497 feature identification. More specifically, the matched filter algorithm was used to identify
498 peaks (full width at half maximum set to 7.5 seconds). Then the peaks were grouped into
499 features using the "peak density" method (52). The area under the peaks was integrated to
500 represent the abundance of features. The retention time was adjusted based on the peak groups
501 presented in most of the samples. To annotate possible metabolites to identified features, the
502 exact mass and MS2 fragmentation pattern of the measured features were compared to the
503 records in HMDB (53) and the public MS/MS database in MSDIAl (54), referred to as MS1
504 and MS2 annotation, respectively. The QC samples were used to control and remove the
505 potential batch effect, t-test was used to compare the features' intensity from spent media with
506 fresh media.

507

508 **Targeted short chain fatty acid (SCFA) measurement**

509

510 The 3-NPH method was used for the quantitation of SCFAs (55). Briefly, 40 μ L of the SM and
511 15 μ L of isotopically labeled standards (ca 50 μ M) were mixed with 20 μ L 120 mM EDC HCl-
512 6% pyridine-solution and 20 μ L of 200 mM 3-NPH HCL solution. After 30 min at 40°C and
513 shaking at 1000 rpm using an Eppendorf Thermomix (Eppendorf, Hamburg, Germany), 900
514 μ L acetonitrile/water (50/50, v/v) was added. After centrifugation at 13000 U/min for 2 min
515 the clear supernatant was used for analysis. The same system as described above was used. The
516 electrospray voltage was set to -4500 V, curtain gas to 35 psi, ion source gas 1 to 55, ion source
517 gas 2 to 65 and the temperature to 500°C. The MRM-parameters were optimized using
518 commercially available standards for the SCFAs. The chromatographic separation was
519 performed on a 100 \times 2.1 mm, 100 Å, 1.7 μ m, Kinetex C18 column (Phenomenex,
520 Aschaffenburg, Germany) column with 0.1% formic acid (eluent A) and 0.1% formic acid in
521 acetonitrile (eluent B) as elution solvents. An injection volume of 1 μ L and a flow rate of 0.4
522 mL/min was used. The gradient elution started at 23% B which was held for 3 min, afterward
523 the concentration was increased to 30% B at 4 min, with another increase to 40%B at 6.5 min,
524 at 7 min 100% B was used which was held for 1 min, at 8.5 min the column was equilibrated
525 at starting conditions. The column oven was set to 40°C and the autosampler to 15°C. Data
526 acquisition and instrumental control were performed with Analyst 1.7 software (Sciex,
527 Darmstadt, Germany).

528

529 **Dynamic metabolic profiling of bacterial supernatants**

530 All chemicals were purchased from Sigma Aldrich at the highest purity available. 50 μ l of the
531 supernatants were spiked with 100 nmol sodium pyruvate- $^{13}\text{C}_3$ and 250 nmol norvaline as

532 internal standards, afterwards the samples were dried under a gentle stream of nitrogen. For
533 derivatization 100 μ l of a methoxyamine hydrochloride solution (10mg/1 ml pyridine) were
534 added and the sample was shaken at 40°C for 90 min. Afterwards 100 μ l of MTBSTFA (N-
535 (tert-butyl)dimethyl-silyl)-N-methyl-trifluoroacetamide containing 1% tert-butyl-dimethyl-
536 silylchlorid) was added and the sample was heated at 70°C for 45 min. GC-MS-analysis was
537 performed with a QP2010 Plus or Ultra gas chromatograph/mass spectrometer (Shimadzu)
538 equipped with a fused silica capillary column (Equity TM-5; 30 m \times 0.25 mm, 0.25 μ m film
539 thickness; SUPELCO) and a quadrupole detector working with electron impact ionization at
540 70 eV. An aliquot of the derivatized samples was injected in 1:5 split mode at an interface
541 temperature of 260°C and a helium inlet pressure of 70 kPa. After sample injection, the column
542 was first kept at 60°C for 3 min and then developed with a temperature gradient of 10°C min⁻¹
543 to a final temperature of 300°C. This temperature was held for further 3 min.

544 Pyruvate results were calculated relative to the pyruvate-¹³C₃ standard (R_t 12.2 min), whereas
545 all other metabolites were calculated relative to norvaline (R_t 17.7 min).

546 For qualitative sugar analysis 50 μ l of the medium were dried under a gentle stream of nitrogen.
547 For derivatization 100 μ l of a methoxyamine hydrochloride solution (10mg/1 ml pyridine) were
548 added and the sample was shaken at 40°C for 90 min. Afterwards 100 μ l of MSTFA (N-methyl-
549 N (trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane) was added and the
550 sample was heated at 50°C for 45 min. GC-MS-analysis was performed as described above.
551 Glucose, fructose, galactose, mannose and trehalose were confirmed with standard solutions.

552 **Spot assays**

553 Bacterial cultures and subcultures were grown for 24 hours each in 10ml AF medium at 37°C
554 under anaerobic conditions without shaking. Monocultures were diluted to OD_{600nm} 0.1 in fresh
555 AF medium. To generate a dense bacterial lawn, monoculture inocula were diluted in LB soft
556 agar to OD_{600nm} 0.01 and poured on a AF medium agar plate. After drying all respective other
557 bacteria were spotted onto the bacterial lawn in duplicates in a volume of 5 μ l with OD_{600nm}
558 0.1. Plates were incubated at 37°C for 24h under anaerobic conditions.

559 **Co-culture experiments**

560 Monoculture inocula were prepared from a previous culture and subculture and were diluted to
561 OD_{600nm} 0.1 in fresh AF medium. Following, pairwise co-cultures were generated by pooling
562 diluted inocula in a 1:1 ratio. From each co-culture 150 μ l were set aside for pH measurements
563 and determination of initial relative abundances (timepoint 0h). The remaining co-cultures
564 were diluted 1:10 to OD_{600nm} 0.01 and pipetted into a round bottom 96-well plate (Nunc).
565 Growth measurements were performed as described above for 72 hours. Samples for qPCR
566 analysis and pH measurements were taken every 24 hours and the co-cultures were serially
567 diluted 1:100 into 150 μ l fresh AF medium in a new 96-well round bottom plate to allow
568 communities to approach a steady state composition over ~25 bacterial generations.

569 **DNA extraction**

570 DNA extraction was performed in the 96-well format using the PureLink™ Pro 96 genomic
571 DNA Kit (Invitrogen) following the corresponding lysis protocol for Gram positive bacterial
572 cells using lysozyme and proteinase K.

573 **Quantitative PCR of bacterial 16S rRNA genes**

574 Quantitative PCR was performed as described previously (23). Strain-specific 16S rRNA
575 primers and hydrolysis probes were used for amplification. Standard curves were determined
576 using linearized plasmids containing the 16S rRNA gene sequence of the individual strains.

577 The standard specific efficiency was then used for absolute quantification of 16S rRNA gene
578 copy numbers of individual strains.

579 **Determination of co-culture outcomes**

580 Quantitative 16S rRNA copy numbers from the measurement endpoint of three independent
581 co-culture experiments were determined by qPCR. Co-culture outcomes (positive, neutral or
582 negative) were determined by calculating the individual abundance ratio for each strain in co-
583 culture relative to monoculture. Therefore, the strain specific absolute abundance at 72h in all
584 pairwise co-cultures was divided by the strain specific absolute abundance at 72h in
585 monoculture ($r_{i,bm} = \frac{m_{i,co}(t72h)}{m_{i,mono}(t72h)}$) for every individual experiment. Following, the mean
586 abundance ratio from all individual experiments (n=3 per strain combination) was calculated.
587 Significance was determined using a two-sided t-test.

588 **Community experiments**

589 Monoculture inocula were prepared from a previous culture and subculture and were diluted to
590 OD_{600nm} 0.1 in fresh AF medium. Following, the community inoculum with equivalent ratios
591 of all 12 strains was generated from this dilution. The inoculum was distributed to ten cell
592 culture flasks, thereby diluting the inoculum 1:10 to 10ml fresh AF medium, resulting in a
593 starting OD_{600nm} 0.01. Cell culture flasks were incubated at 37°C without shaking under
594 anaerobic conditions. Every 24h for 10 days samples were taken for qPCR analysis, OD
595 measurement and pH measurement and cultures were diluted 1:100 in 10ml fresh-AF medium.

596 **Malate uptake measurements**

597 Uptake of ¹⁴C-malate by *E. faecalis* KB1 was determined in principle as previously described
598 (56). Briefly, *E. faecalis* was grown anaerobically in LB medium with 40 mM malate and
599 harvested in mid-log phase. Cells were centrifuged, washed twice with 50 mM Tris-HCl buffer
600 (pH 7.4) containing 10 mM MgCl₂ and resuspended in 50 mM Tris-maleate buffer (pH 7.2)
601 containing 5 mM MgCl₂, thereby adjusting the cell suspension to an OD₆₀₀ of 10. For transport
602 assays, this cell suspension was diluted 1:10 with 50 mM Tris-maleate buffer (pH 7.2)
603 containing 5 mM MgCl₂ and 1 % (w/v) peptone. Uptake of ¹⁴C-malate (55 mCi.mmol⁻¹
604 [Biotrend]) was measured at a total substrate concentration of 10 μM at 18° C. At various time
605 intervals, transport was terminated by the addition of stop buffer (100 mM potassium phosphate
606 buffer, pH 6.0, 100 mM LiCl), followed by rapid filtration through membrane filters (MN gf-
607 5 0.4 μm; Macherey-Nagel). The filters were dissolved in 5 ml of scintillation fluid (MP
608 Biomedicals), and radioactivity was determined in a liquid scintillation analyzer
609 (PerkinElmer). Total protein content of *E. faecalis* cells in relation to OD₆₀₀ was determined
610 with a suspension of lysed cells as described before (57).

611

612 **Data analysis and Figures**

613 Data was analyzed using R Studio (Version 1.4.1103). Heatmaps were generated using the R
614 *heatmap* package (<https://github.com/raivokolde/heatmap>). Plots were generated using the
615 R *ggplot2* package (58) and *ggpubR* package (<https://github.com/kassambara/ggpubr>). Figures
616 were partly generated using BioRender (<https://biorender.com>) and Adobe Illustrator CC
617 (Adobe Inc.).

618

619 **Figure Legends**

620 **Figure 1: Growth analysis of OMM¹² strains in spent media experiments**

621 (A) Phylogenetic tree for bacteria of the OMM¹² consortium based on the individual 16S rRNA
622 genes. The consortium represents the five major phyla of the murine gastrointestinal tract:
623 Firmicutes (green), Bacteroidetes (orange), Verrucomicrobia (purple), Actinobacteria (blue)
624 and Proteobacteria (red). (B) Flowchart depicting spent culture medium (SM) preparation by
625 growing bacterial monocultures in fresh AF medium for 20h. Culture supernatants were sterile-
626 filtered, samples for pH measurements and MS were collected, and the SM was used as culture
627 medium for the growth of all respective other strains. After growth of the individual strains in
628 the specific SM, pH of the double spent medium (DSM) was determined. Differences in pH
629 were then analyzed by calculating the corresponding $\Delta\text{pH}_{\text{SM}}$ and $\Delta\text{pH}_{\text{DSM}}$. (C) Monoculture
630 growth in SM resulted in mostly decreased area under the growth curve (AUC) values in
631 comparison to fresh AF medium, which was analyzed by calculating the inhibition factor d_{AUC} .
632 d_{AUC} was calculated from the mean AUC of three independent experiments relative to the mean
633 AUC in fresh medium. (D) The mean pH of all SM (center of circles) and DSM (outer tiles)
634 after growth of the individual strains in fresh medium and the respective SM was determined
635 from three independent experiments. (E) Spot assays to determine production of antibacterial
636 agents. All bacterial strains of the OMM¹² consortium were spotted onto a bacterial lawn of all
637 the respective other strains. Inhibition zones were observed for *B. animalis* YL2, *F. plautii*
638 YL31, *E. clostridioformis* YL32, *C. innocuum* I46 and *L. reuteri* I49 when *E. faecalis* KB1
639 was spotted. No inhibition zone was seen for *E. faecalis* KB1 on itself. AF medium with *E.*
640 *faecalis* KB1 spotted is shown as control.

641

642 **Figure 2: Overlap of substrate depletion profiles between individual OMM¹² strains**

643 (A) Depletion profiles of substrates after bacterial growth to stationary phase in AF medium
644 were determined by untargeted MS from three independent experiments. All metabolomic
645 features (rows) that significantly decreased ($p < 0.05$ compared to fresh media) compared to
646 fresh medium for at least one of the twelve strains are shown in red. Dark-red indicates strong
647 depletion, while white indicates no depletion of the metabolomics feature. Hierarchical
648 clustering of strain specific profiles as well as metabolomic features reveal profile similarities
649 between phylogenetically similar strains. (B) Bar plot showing the total number of significantly
650 ($p < 0.05$ compared to fresh media) depleted metabolomic features in AF medium for the
651 individual strains. (C) Pairwise overlap in depletion profiles in percent of the total number of
652 individually depleted metabolomic features. (D) Euler diagram depicting total number of
653 depleted metabolomic features and overlap within the consortium. Size of the ellipse denotes
654 number of depleted features, size of overlap between ellipses denotes number of features that
655 are shared when comparing the individual profiles.

656

657 **Figure 3: Metabolic potential of the OMM¹² strains**

658 (A) OMM¹² genomes were screened for a hand-curated set of key enzymes to determine the
659 strains' potential to use a diverse range specific substrates, metabolic pathways and release
660 fermentation end products. A potential substrate and pathway utilization was considered
661 positive (green) if one of the associated KO's was found in the respective genome (**SI data**
662 **table**). Consequently, positive hits do not indicate completeness of the pathway. If none of the
663 associated KO's was found in the respective genome, the potential substrate and pathway
664 utilization was considered negative (grey). Metabolites and pathways were sorted by functional

665 groups. **(B)** By combining metabolomics data (MS, **Fig. S10, S11**) with genome-based
666 information on the presence of key enzymes, broad-scale draft metabolic models of the
667 individual OMM¹² strains were generated (supplementary text C, **Fig. S12**). Here, the models
668 for strains *E. faecalis* KB1, **(C)** *B. caecimuris* I48, **(D)** *B. coecoides* L58 and **(E)** *F. plautii*
669 YL31 are shown. Models of remaining strains of the consortium are shown in **Fig. S12**.
670 Experimentally confirmed substrates, products or enzymes are shown in black. Hypothetical
671 substrates, products or enzymes are shown in grey.

672

673 **Figure 4: Pairwise cultures of the OMM¹² strains**

674 **(A)** OMM¹² pairwise strain combinations (12 monocultures, 66 co-cultures) were cultured in a
675 1:1 ratio in fresh AF medium over the course of 72 hours and growth, pH and relative
676 abundance was monitored over time in three independent experiments. Growth at OD_{600nm} and
677 pH is shown as mean with the corresponding standard deviation (grey), relative abundance
678 over time is shown exemplary for one of the three experiments. Examples of how growth curves
679 develop with changing relative abundances is shown for the co-culture of *T. muris* YL45 and
680 *L. reuteri* I49 **(B)**. Starting with a OD_{600nm} ratio of approximately 1:1, final mean OD_{600nm}
681 values after the first two turnovers are low, corresponding to YL45 dominating the co-culture.
682 After 48h, *L. reuteri* I49 resumes growth and final OD values increase. With *L. reuteri* I49
683 dominating the community in the end, the growth curve as well resembles *L. reuteri* I49
684 monoculture growth. Similarly, pH values reflect changes in co-culture structure, as can be
685 observed e.g. in the co-culture of *C. innocuum* I46 and *B. coecoides* YL58 **(C)**. In monoculture,
686 *C. innocuum* I46 does not strongly acidify its environment, while YL58 acidifies the culture
687 supernatant to around pH 6.0. In accordance with this observation, pH values of the co-culture
688 supernatant only drop with increasing dominance of YL58 in the co-culture.

689

690 **Figure 5: Transferring pairwise interactions to the community level**

691 **(A)** By comparing the mean bacterial abundance from three independent experiments in co-
692 culture to the mean abundance in monoculture, a pairwise interaction matrix was generated.
693 Interactions where the individual abundance in co-culture significantly (t-test, p < 0.05)
694 increased are indicated with (+), interactions where it significantly decreased are indicated with
695 (-) and interaction where the abundance did not change in comparison to monoculture growth
696 were indicated with (0). **(B)** Using a serial passaging batch culture setup, the OMM¹²
697 community composition was analyzed after ten days of serial dilutions by comparing the
698 relative strain abundances of ten replicates (F1-F10) with the inoculum. **(C)** To study the
699 influence of *E. faecalis* KB1 on community composition, a OMM¹¹-KB1 dropout community
700 was constructed and relative abundance at day ten was analyzed for ten replicates (F1-F10).
701 **(D)** Based on SM experiments, pairwise co-culture and community experiments a predation
702 interaction mechanism between strains *C. innocuum* I46 and *E. faecalis* KB1 is proposed. We
703 hypothesize, that KB1 produces a bacteriocin inhibiting I46, and benefits from cross-feeding
704 on I46 derived metabolites. **(E)** Potentially cross-fed metabolites were determined by
705 comparing SM profiles (determined by untargeted MS) of KB1 and I46 for metabolites that are
706 strongly produced by I46 and consumed by KB1. Verified annotations are shown in green,
707 potential annotations are shown in black and not annotated compounds are shown in grey as
708 the corresponding feature identification numbers. **(F)** Time course of malate uptake by whole
709 cells of *E. faecalis* KB1. Rates of ¹⁴C-malate uptake were measured at a final malate
710 concentration of 10 μM at 18° C. Standard deviations are estimated from three biological
711 replicates.

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721

722 **Author contributions**

723 B.S., A.S., K.J. and A.S.W. conceived and designed the experiments. A.S.W., A.v.S., A.B.,
724 A.C.D.R., L.R., S.G., K.K. and C.H. performed the experiments. A.S.W., A.B., A.C.D.R., L.R.,
725 C.M. C.H., K.K., S.G. and P.M. analyzed the data. P.M., K.K., C.M., C.H., K.J. and W.E.
726 contributed materials/ analysis tools. B.S. coordinated the project. B.S., L.M.J. and A.S.W.
727 wrote the original draft and all authors reviewed and edited the draft manuscript.

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729 **Competing interests**

730 The authors declare no competing financial interests.

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732 **References**

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Figure 1

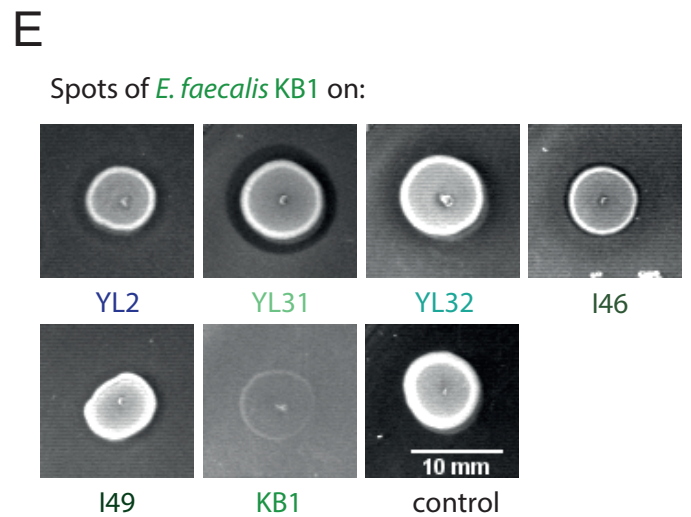
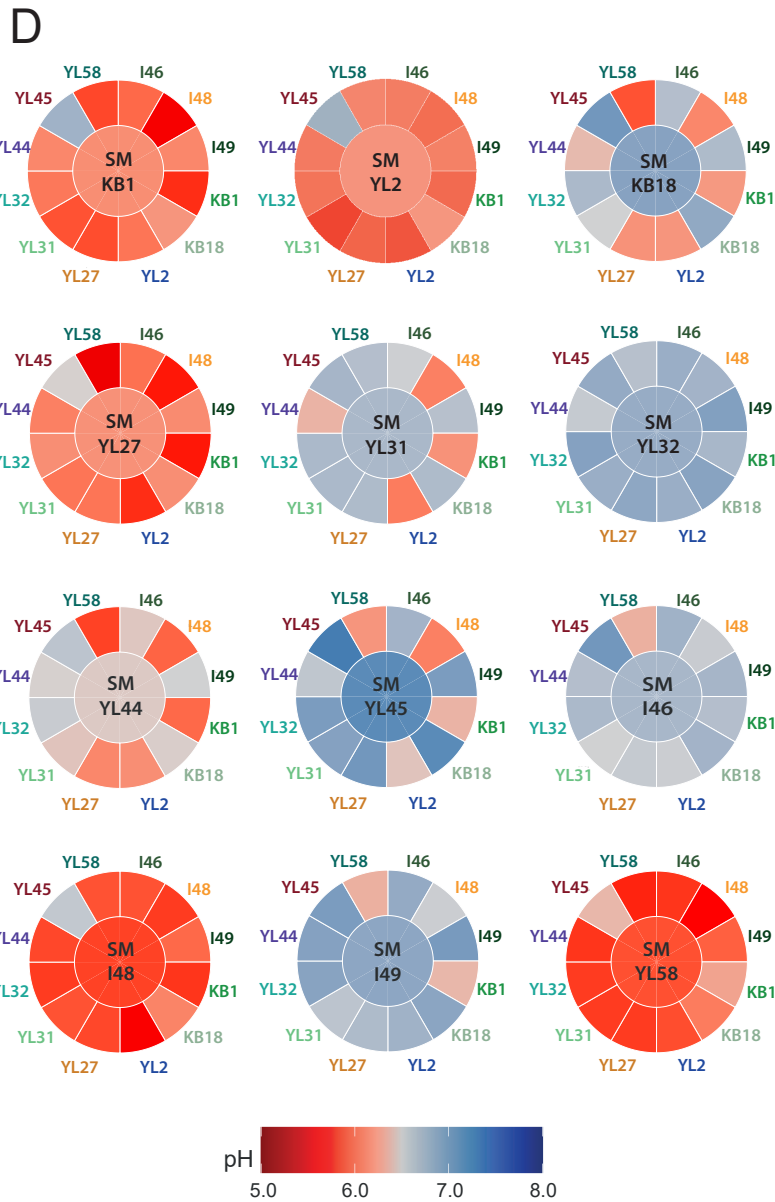
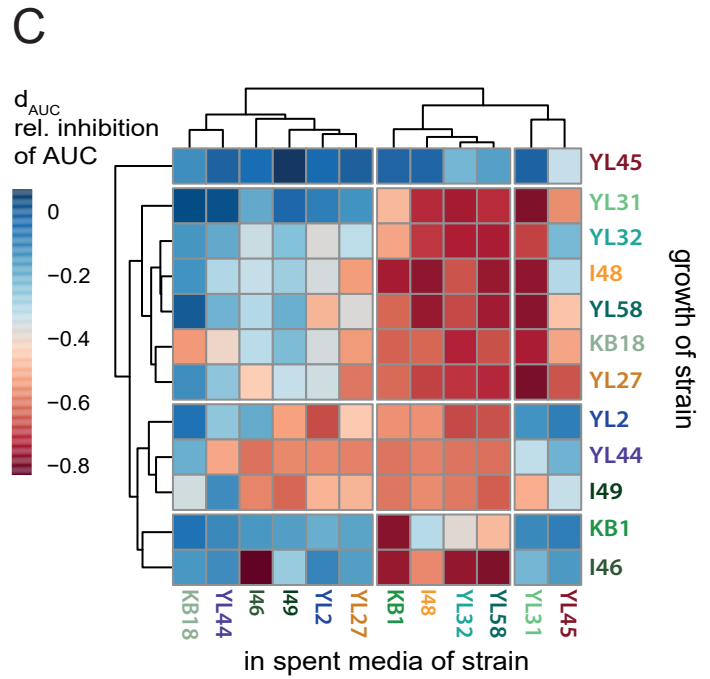
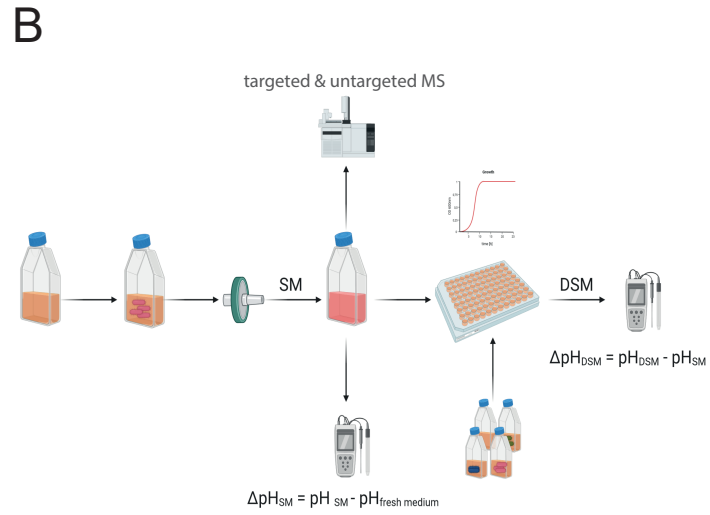
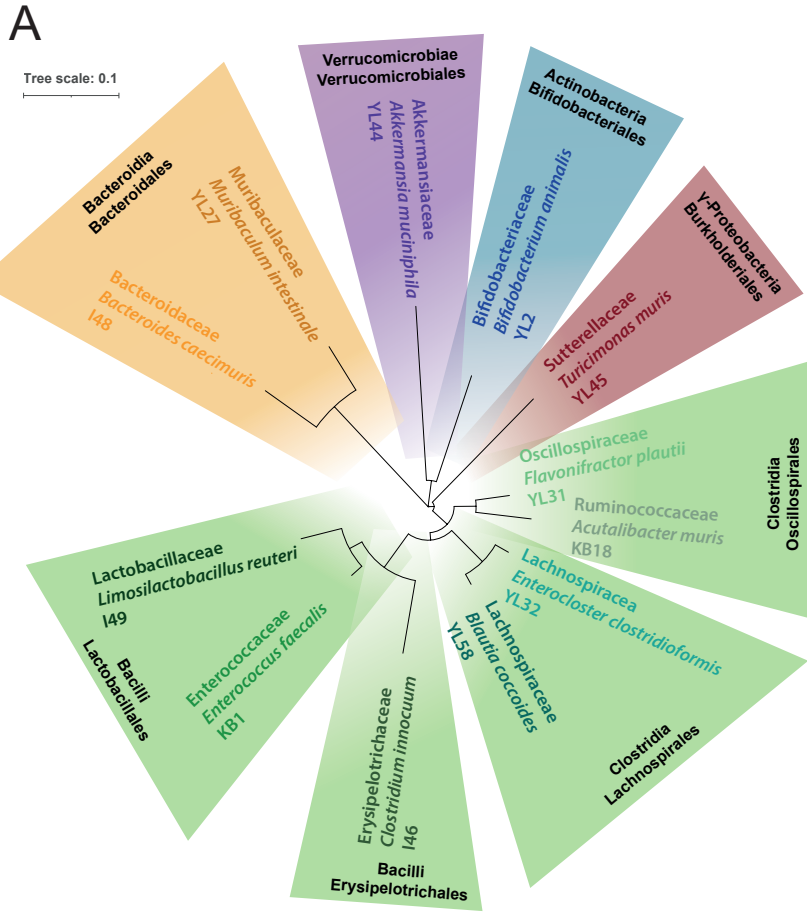


Figure 2

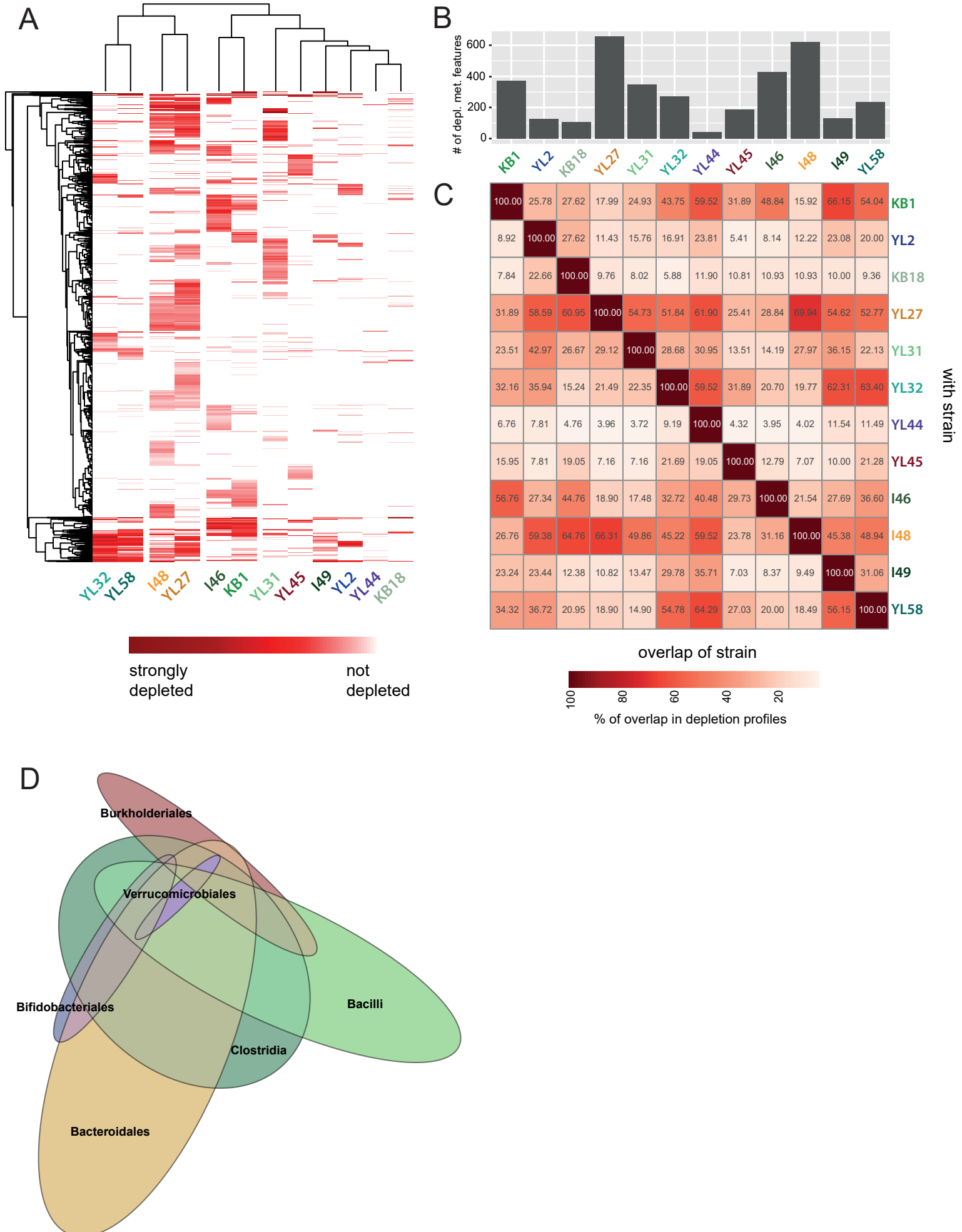
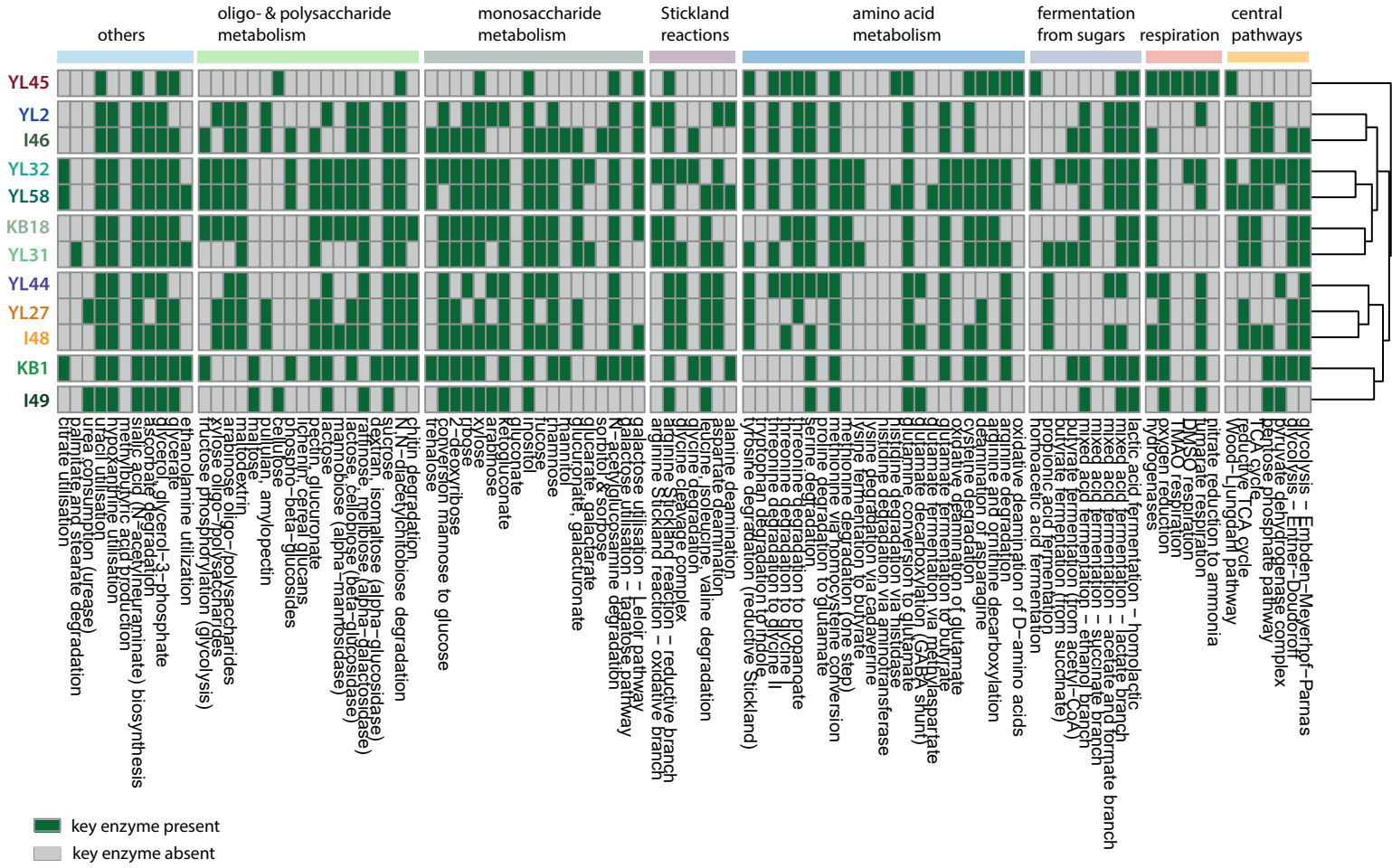


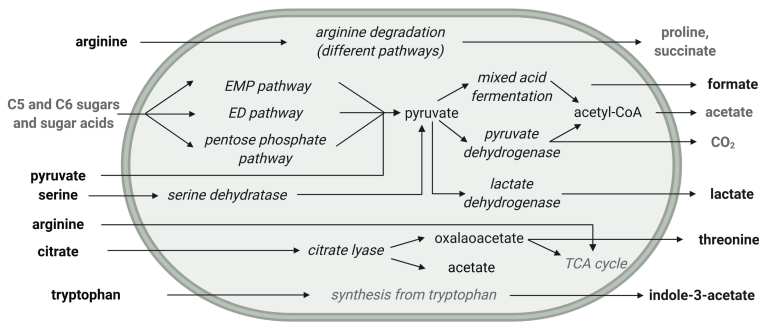
Figure 3

A



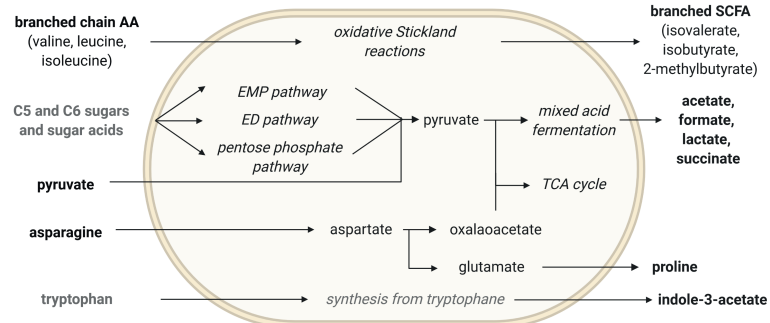
B

Enterococcus faecalis KB1



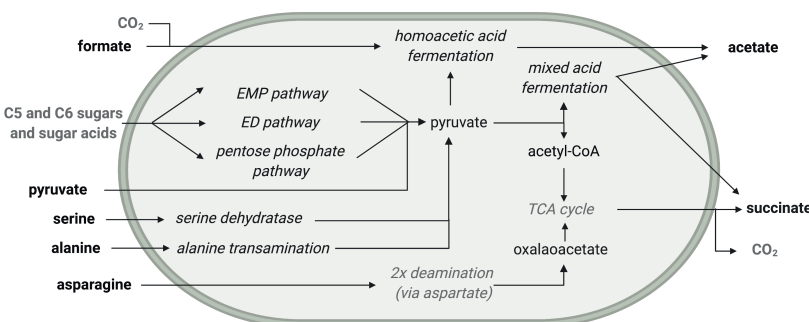
C

Bacteroides caecimuris I48



D

Blautia coccoides YL58



E

Flavonifractor plautii YL31

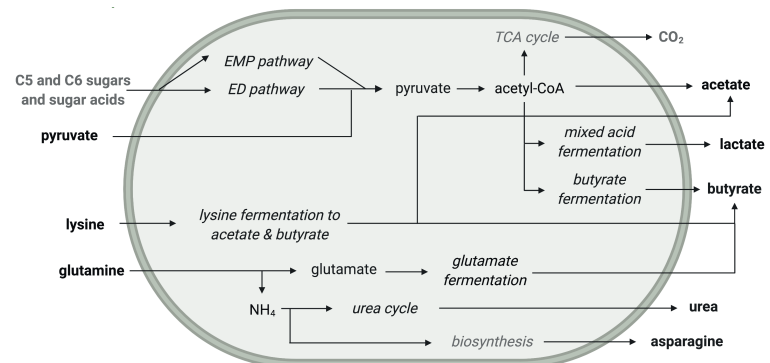
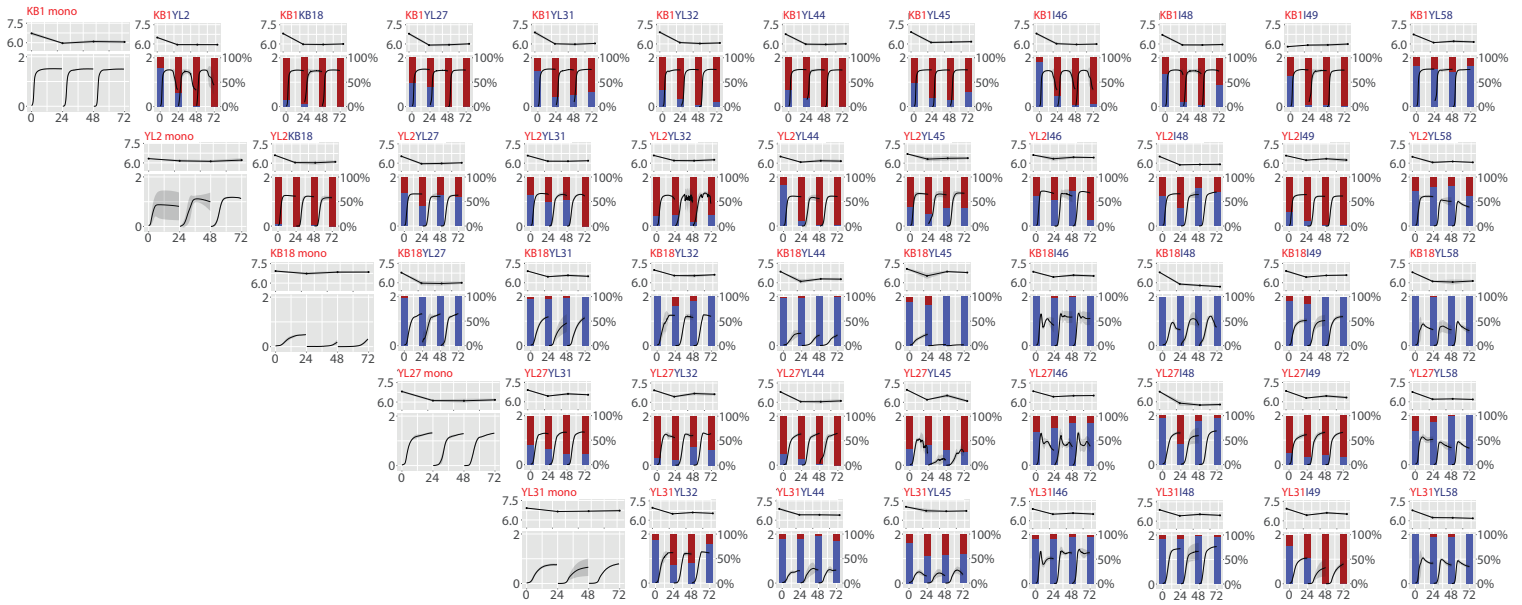
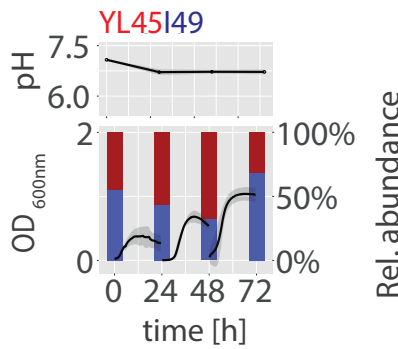


Figure 4

A



B



C

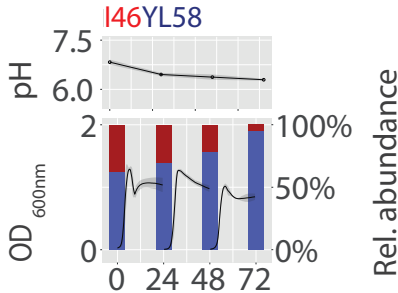
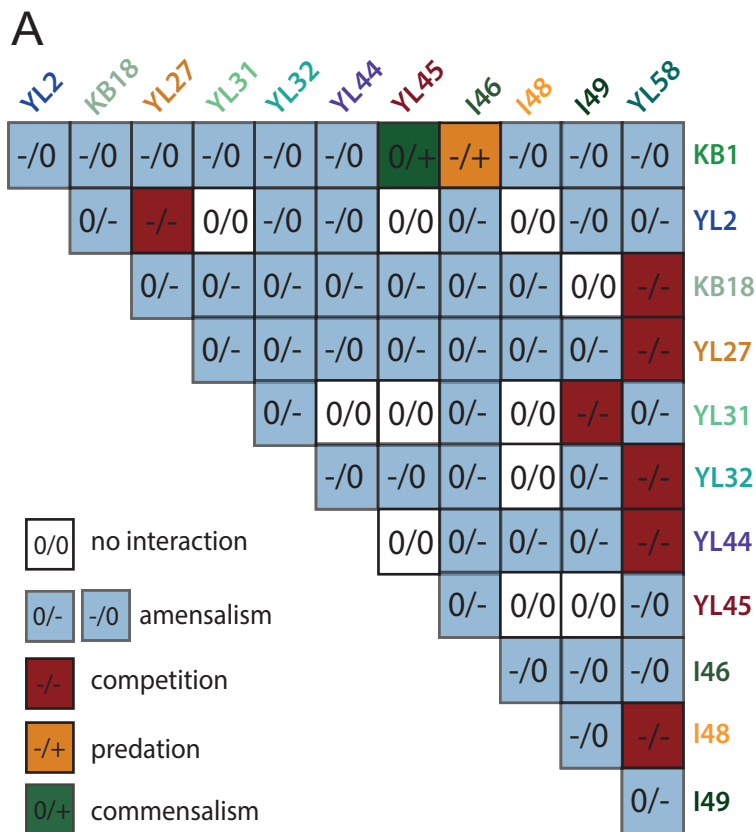


Figure 5



D Hypothesised interaction mechanism:

