In vitro interaction network of a synthetic gut bacterial community

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A key challenge in microbiome research is to predict the functionality of microbial communities based on community membership and (meta-)genomic data. As central microbiota functions are determined by bacterial community networks, it is important to gain insight into the principles that govern bacteria-bacteria interactions. Here, we focused on the growth and metabolic interactions of the Oligo-Mouse-Microbiota (OMM12) synthetic bacterial community, which is increasingly used as a model system in gut microbiome research. Using a bottom-up approach, we uncovered the directionality of strain-strain interactions in mono- and pairwise co-culture experiments as well as in community batch culture. Metabolic network reconstruction in combination with metabolomics analysis of bacterial culture supernatants provided insights into the metabolic potential and activity of the individual community members. Thereby, we could show that the OMM12 interaction network is shaped by both exploitative and interference competition in vitro in nutrient-rich culture media and demonstrate how community structure can be shifted by changing the nutritional environment. In particular, Enterococcus faecalis KB1 was identified as an important driver of community composition by affecting the abundance of several other consortium members in vitro. As a result, this study gives fundamental insight into key drivers and mechanistic basis of the OMM12 interaction network in vitro, which serves as a knowledge base for future mechanistic in vivo studies.

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INTRODUCTION

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

A multitude of different approaches has been used to characterize the ecological networks of microbial communities. Function-related patterns in native microbial communities can be identified by systems biology approaches, combining metagenomics, metatranscriptomics, and metabolomics analyses [14]. Together with methods based on stable isotope probing, microorganisms with specific metabolic properties can be identified [15]. Potentially interacting species may be predicted from co-occurrence analysis supported by genome-guided metabolic modeling [16–18]. To experimentally verify the key ecological, structural, and functional role of certain species in driving community structure and function, synthetic microbial consortia provide several advantages over native communities. As they are well-characterized, scalable, and experimentally tractable, these systems are increasingly used to gain a mechanistic understanding of gut microbial ecology [19–22].

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The Oligo-Mouse-Microbiota (OMM12) is a synthetic bacterial community, which stably colonizes mice and provides colonization resistance against enteropathogen infection [23–26]. The OMM12 comprises twelve bacterial species (Enterococcus faecalis KB1, Limosilactobacillus reuteri I49, Bifidobacterium animalis YL2, Clostridium innocuum I46, Blautia coccoides YL58, Enteroclostridium clostridioformis YL32, Flavonifractor plautii YL31, Acutalibacter muris KB18, Bacteroides caecimuris I48, Mureubaculum intestinale YL27, Akkermansia muciniphila YL44 and Turicimonas muris YL45), representing the five major eubacterial phyla in the murine gastrointestinal tract [27] (Fig. 1A). The model is openly available for non-commercial use [28], and is therefore increasingly employed in preclinical microbiome research [29–32]. So far, little is known about the system’s ecology and metabolic capabilities, both of which are factors that determine assembly, population dynamics, and bacterial community functionality. Therefore, we aimed for a comprehensive exploration of the metabolic potential (i.e., substrates, metabolism, and end products) and interactions...
between individual members of the OMM$^{12}$ consortium. We employed a bottom-up approach connecting outcomes of mono- and pairwise co-culture experiments with observations from complex communities in in vitro batch culture. Furthermore, we combined metabolomics analysis of spent culture supernatants with genome-informed pathway reconstruction and generated draft metabolic models of the OMM$^{12}$ consortium. Overall, we find that the majority of in vitro strain-strain interactions are amensalistic or competitive, which may be due to the environmental conditions in rich culture media. In accordance, bacteriocin production and substrate overlap between the individual strains was correlated with negative strain-strain interaction in vitro. Together, this work identified key interaction patterns among OMM$^{12}$ strains relevant in community assembly and functionality.

## RESULTS

### Probing directional interactions of OMM$^{12}$ strains using spent culture media

To characterize directional interactions of the OMM$^{12}$ consortium members, we chose an in vitro approach to explore how the bacterial strains alter their chemical environment by growth to late stationary phase.

Growth of the individual monocultures in a rich culture medium that supports growth of all members (AF medium, Methods, Table S1) was monitored over time (Fig. S1; SI data table 1) and growth rates (Table S2) were determined. Strains were grouped by growth rate (GR) into fast growing strains (GR $> 1$ h$^{-1}$) and slow growing strains (GR $< 1$ h$^{-1}$). прич

**Fig. 1** Growth analysis of OMM$^{12}$ strains in spent media experiments. (A) Phylogenetic tree for bacteria of the OMM$^{12}$ consortium based on the individual 16S rRNA genes. The consortium represents the five major phyla of the murine gastrointestinal tract: Firmicutes (green), Bacteroidetes (orange), Verrucomicrobia (purple), Actinobacteria (blue) and Proteobacteria (red). (B) Flowchart depicting spent culture medium (SM) preparation by growing bacterial monocultures in fresh AF medium for 20 h. Culture supernatants were sterile-filtered, samples for pH measurements and mass spectrometry were collected, and the SM was used as culture medium for the growth of all respective other strains. After growth of the individual strains in the specific SM, pH of the double spent medium (DSM) was determined. Differences in pH were then analyzed by calculating the corresponding $\Delta$pH$_{SM}$ and $\Delta$pH$_{DSM}$. (C) Monoculture growth in SM resulted in mostly decreased area under the growth curve (AUC) values in comparison to fresh AF medium, which was analyzed by calculating the inhibition factor $d_{AUC}$. $d_{AUC}$ was calculated from the mean AUC of three independent experiments relative to the mean AUC in fresh medium. (D) The mean pH of all SM (center) and DSM (outer tiles) after growth of the individual strains in fresh medium and the respective SM was determined from three independent experiments. Absolute values are available in SI data table 1. (E) Spot assays to test for production of antibacterial compounds. All bacterial strains of the OMM$^{12}$ consortium were spotted onto a bacterial lawn of all the respective other strains. Inhibition zones were observed for B. animalis YL2, F. plautii YL31, E. clostridiformis YL32, C. innocuum I46 and L. reuteri I49 when E. faecalis KB1 was spotted. No inhibition zone was seen for E. faecalis KB1 on itself. AF medium with E. faecalis KB1 spotted is shown as control.

Individual pH profiles as indicators for niche modification

The pH of the culture medium after growth to stationary phase can be used as a measure for the extent of strain-specific environmental modification [$11$] and may partly explain inhibition of bacterial growth in a SM. Therefore, we determined the pH of the individual SM before and after (double spent media; DSM) growth of all OMM$^{12}$ strains (Fig. 1B; SI data table 1). From these values, we defined the $\Delta$pH for every strain after growth in fresh medium ($\Delta$pH$_{SM}$) and in all SM ($\Delta$pH$_{DSM}$) by analyzing the strength (difference of pH values) and direction (more acidic or more alkaline) of the pH change (Fig. 1D). After growth in fresh AF medium with neutral pH of 7.0, the OMM$^{12}$ strains showed different degrees of $\Delta$pH$_{SM}$. While E. faecalis KB1, B. animalis YL2, M. intestinale YL27, B. caecimuris I48 and B. coccoideis YL58 distinctly acidified the medium (pH$_{SM}$ < 6.2), the growth of the other strains resulted in either slightly more alkaline or nearly neutral medium. Correlating inhibition of growth in a SM ($d_{AUC}$) with the mean pH of the individual SM for each strain revealed that growth inhibition did not directly correlate with the pH. Only strains B. animalis YL2, A. muciniphila YL44 and B. caecimuris I48 showed a significant negative correlation (R $< -0.5$, p $< 0.05$) between growth inhibition and pH (Fig. S4; SI data table 1) with stronger inhibition in more acidic pH ranges. Testing monoculture growth in fresh AF medium with adjusted pH values from pH 5 to pH 7.5 revealed that these strains indeed showed decreased growth rates and lower final OD values in medium with pH $< 6.5$ (Fig. S5; SI data table 1), pH sensitivity was further observed for M. intestinale YL27.

Most interestingly, many strains did not show the same magnitude or direction of alteration in pH when grown in SM of another strain ($\Delta$pH$_{DSM}$) compared to growth in fresh culture medium ($\Delta$pH$_{SM}$). This indicates an altered metabolic behavior of some strains in specific SM environments that differs from metabolic behavior in fresh AF medium (Fig. S6, Supplementary Text A).

### Production of antibacterial compounds by E. faecalis KB1

Growth inhibition in SM (Fig. 1C) may further be explained by the production of antimicrobial compounds. To test for the production of antimicrobial compounds by the OMM$^{12}$ strains, we used a phenotyping approach and performed spot assays on agar plates (Fig. 1E). Inhibition zones were only seen in case of E. faecalis KB1, which produced one or several compounds active against...
Genomic analysis revealed that the strain encodes genes for the production of several bacteriocins (Supplemental Text B), including enterocin L50, an enterococcal leaderless bacteriocin with broad target range among Gram-positive bacteria [33]. All other strain pairs did not show signs of growth inhibition by compound excretion under these conditions, despite the presence of genes for lanthibiotic production in the genome of *B. coccoides* YL58 (determined by antiSMASH) [34]. Although expression of antimicrobial molecules may be induced by specific environmental triggers, which are absent in the monoculture in vitro setting, we concluded that interference competition may only play a role in a subset of pair-wise interactions in AF medium involving *E. faecalis* KB1.
Substrate depletion profiles correlate with growth inhibition in SM
As pH and antimicrobial compounds only partly explained inhibition of growth in SM, we set out to gain more insights into the individual metabolic profiles in our in vitro setting. Therefore, triplicate samples of fresh AF medium and SM were analyzed by a mass spectrometry-based untargeted metabolomics approach (TripleTOF, Methods). Combining positive and negative ionization mode, 3092 metabolomic features were detected in total. From these, 2387 (77.20 %) were significantly altered (t-test, p value < 0.05) by at least one of the twelve strains (Fig. S7). Hierarchical clustering of the metabolomic feature depletion profiles (i.e., substrates used by the bacteria; Fig. 2A) reflects the phylogenetic relationship between the strains (Fig. 1A). Correlating the phylogenetic distance between the individual strains with the number of shared depleted metabolomic features in AF medium (Fig. S8) showed that phylogenetically similar strains of the consortium have a higher substrate overlap than phylogenetically distant strains (R = −0.29, p = 0.017). The total number of metabolomic features that are depleted from AF medium greatly varies for the different strains, ranging from over 600 depleted features for M. intestinale YL27 to only 42 for A. muciniphila YL44 (Fig. 2B). The number of metabolomic features overlapping with other OMM12 strains’ features relative to the strains’ total set of depleted features was determined (Fig. 2C). Phyllogenetically related strains like E. clostридiumformis YL32 and B. cocoides YLS8 or M. intestinale YL27 and B. caecimuris I48 share over 50% of depleted metabolomic features each, suggesting a strong substrate overlap in AF medium. Visualizing the extent of overlap between substrate depletion profiles reveals that Bacteroidales, Clostridia and Bacilli strains of the consortium dominate with the highest number of commonly depleted substrates in AF medium (Fig. 2D).

Correlating the growth inhibition in SM (dAUC) with the pairwise overlap in depletion profiles (Fig. 2C) revealed that a larger overlap is correlated with a stronger growth inhibition in the corresponding SM (R = −0.46, p = 3.1E−08, Fig. S9). This is illustrated by A. muciniphila YL44, which used only a low number of substrates from the AF medium (Fig. 2B) and the SM of which had only little effect on the growth of the other strains of the consortium (Fig. 1C). On the other hand, the strain’s growth itself was strongly reduced in the SM of most other consortium members (Fig. 1C, S2), which depleted a large spectrum of metabolomic features including those used by A. muciniphila YL44 (Fig. 2C).

Genome-informed metabolic potential of the OMM12 consortium
To gain insights into metabolic properties of the OMM12 strains, we reconstructed genome-scale metabolic models using gapseq [35] (SI data file). The initial metabolic models were curated by screening for metabolic pathways and transporter proteins and filling of missing reactions (gap-filling). From the genome-based metabolic models, we derived the presence and absence of metabolic pathways for central carbon metabolism (e.g., fermentation pathways, respiration), amino acid metabolism, and utilization of specific substrates, for the individual strains using MetaCyc pathways [36] (Fig. 3A, Fig. S10, SI data table 2). Furthermore, the presence of specific substrate transporters was determined (Fig. S11). Hierarchical clustering of the genome-informed metabolic potential (Fig. 3A) reflected their phylogenetic relationship in many instances. Generally, a high diversity of central and fermentation pathways was found among the consortium members. Moreover, enzymes for the degradation of amino acids (e.g., aspartate, glutamate, serine, and cysteine) are highly prevalent among consortium members. Correspondingly, systems for amino acid transport were especially prevalent among all strains of the consortium (Fig. S11).

Metabolite production and fermentation pathways of the OMM12 strains in AF medium
To verify metabolites and fermentation products produced and consumed by the individual strains of the consortium in the given in vitro conditions, SM were analyzed using different mass spectrometry approaches (Fig. S12, S13). The combination of experimentally obtained insights and genome-based information on the presence of pathways was used to generate sketch drawings to visualize basic metabolic properties of the individual OMM12 community members (Fig. 3B, C, S14).

To confirm if fermentation pathways identified by genomics were active under in vitro conditions, short chain fatty acid (SCFA) production and consumption were analyzed (Fig. S12A). As observed for the SM metabolic profiles (Fig. S7), hierarchical clustering revealed that closely related bacteria showed similar SCFA production and consumption profiles. Both Bacteroidales strains produced acetic acid, succinic acid as well as branched-chain fatty acids. Both Lachnospiraceae strains generated high amounts of acetic acid. Butyric acid is produced by strains F. plautii YL31 and C. innocuum I46, the latter also being the only strain of the consortium excreting valeric acid and caproic acid. Of note, F. plautii YL31 also consumed lysine, indicating the ability to produce butyric acid from lysine, which was supported by the presence of the pathway for lysine fermentation to acetate and butyrate.

Formic acid was produced by several strains and consumed by T. muris YL45 and B. cocoides YLS8. B. cocoides YLS8 encodes genes for a CO dehydrogenase/acetyl-CoA-synthase, the key enzyme of the Wood-Ljungdahl pathway (reductive acetyl-CoA pathway). Formic acid can be processed via this pathway to acetyl-CoA. As another prominent example of bacterial fermentation, lactate production was confirmed (Fig. S12C) for E. faecalis KB1, B. animilis YL2, F. plautii YL31, C. innocuum I46, E. clostридiumformis YLS32 and L. reuteri I49, all of which harbor the pathway for lactate formation.
By quantifying amino acid levels we could show that Bacteroidales and Lachnospirales strains exhibited similar amino acid depletion and production profiles. In SM of strains *M. intestinale* YL27 and *B. caecimuris* I48, elevated levels of a diverse range of amino acids including glutamic acid, histidine, methionine, proline and phenylalanine were detected. Lachnospirales strains showed increased levels of isoleucine, tryptophan and valine, while alanine was especially depleted by *B. coccoides* YL58. Other strains of the consortium showed specific depletion of single amino acids, e.g., *F. plautii* YL31 strongly depleted lysine and glutamic acid, while *E. faecalis* KB1 depleted serine.
Growth of OMM12 strains in pairwise co-culture

Next, we performed a set of experiments to characterize strain-strain interactions in the dynamic community-dependent context. We first analyzed direct competition of all strains in pair-wise co-cultures over the course of 72 h, with serial dilutions every 24 h. While growth was monitored continuously by OD 600 nm, cultures over the course of 72 h, with serial dilutions every 24 h. The growth curves of most co-cultures, as well as supernatant pH differed from the corresponding strain-specific samples for pH measurements and qPCR analysis were taken while growth was monitored continuously by OD 600 nm.

The ISME Journal (2022) 16:1095 – 1109

Fig. 3 Metabolic potential of the OMM12 strains. (A) OMM12 metabolic models were reconstructed using gapseq [35] and gapseq output was screened for a hand-curated set of pathways to determine the strains’ potential to use a diverse range of substrate-specific and central pathways and release fermentation end products. Multiple pathways corresponding to the same function were grouped together according to the MetaCyc pathway database [36] (SI data table 2) and pathway utilization was considered positive (green) if one of the associated pathways was confirmed by gapseq. If none of the associated pathways were found, the potential substrate and pathway utilization was considered negative (grey). Metabolites and pathways were sorted by functional groups. By combining metabolomics data (MS, Fig. S10, S11) with genome-based information, broad-scale metabolic sketches of the individual OMM12 strains were generated (Fig. S12). Here, the models for strains B. caecimuris I48 (B) and B. coccoides YL58 (C) are shown exemplarily. Models of the remaining strains of the consortium are shown in Fig. S12. Experimentally confirmed substrates and products and pathways found by gapseq are shown in black. Hypothetical substrates, products, or pathways are shown in grey.

Community structure of the OMM12 consortium

Next, we set out to investigate if interactions found in co-cultures are transferrable to the strains’ behavior in the complete OMM12 community. To this end, all twelve OMM strains were simultaneously co-cultured in AF medium and serially diluted 1:100 every 24 h into fresh AF medium. Relative abundance of all strains after 10 days was determined by qPCR for ten replicates each in two independent experiments from different inocula (Fig. 4E).

While each of the OMM12 members except E. faecalis KB1 was outcompeted to a very low relative abundance in at least one pair-wise culture (Fig. 4A, B), the majority (10 out of 12) of the consortium members were able to coexist in the complex community up to 10 days (Fig. 4E, Fig. S19A). Replicate communities showed reproducible community structure, even when different inocula were used. Interestingly, F. plautii YL31 dominated the community under these conditions. Furthermore, B. coccoides YL58 and E. faecalis KB1 showed a high relative abundance, which corresponds to their dominant role in SM and co-culture experiments (Figs. 1C, 4A, B). While strains B. animals YL2 and L. reuteri I49 were not detectable at 10 days in all replicates, A. murris YL18 was found in only a few of the communities at 10 days (relative abundance < 1%).

E. faecalis KB1 strongly impacts overall in vitro community composition

Following, we investigated how the absence of E. faecalis KB1, which plays a dominant role in pair-wise interactions, would affect the overall community structure. We generated a “dropout” community including all strains of the OMM12 consortium except E. faecalis KB1 (OMM11-E. faecalis KB1). Compositional analysis revealed increased relative abundance of C. innocuum I46 and B. animals YL2 in the OMM11-E. faecalis KB1 compared to the full OMM12 community (Fig. 4F). In addition, the absolute abundances of strains B. animals YL2, C. innocuum I46, B. coccoides YL58 and B. caecimuris I48 were found to increase significantly (t-test, p < 0.05) in the absence of E. faecalis KB1 (Fig. S19A (M1)). The increase in abundance of B. animals YL2 and C. innocuum I46 may be explained by absent enterocin production or substrate competition by E. faecalis KB1. The latter may also explain increased abundance of B. caecimuris I48 and B. coccoides YL58 in the dropout community. Further, the abundance of F. plautii YL31, E. clostridioformis YL32, A. muciniphila YL44 and T. murris YL45 was found to decrease in the absence of E. faecalis KB1 (Fig. S19A (M1)). This indicates either direct positive effects of E. faecalis KB1 on these strains or indirect effects that occur through the overall shift in OMM11-E. faecalis KB1 community composition compared to the OMM12 consortium.
Fig. 4  Transferring pairwise interactions to the community level. (A) OMM\textsuperscript{12} pairwise strain combinations (12 monocultures, 66 co-cultures) were cultured in a 1:1 ratio in fresh AF medium over the course of 72 h. Mean absolute abundance (normalized 16S rRNA gene copies determined by qPCR) after 72 h was determined. By comparing the mean bacterial abundance from three independent experiments in co-culture to the mean abundance in the corresponding monoculture, the factor $r_{\text{bm}}$ was determined, as a measure of how successful a strain can grow in co-culture relative to monoculture after 72 h is shown. A ratio $r_{\text{bm}} = 1$ indicates no change in absolute abundance in the co-culture compared to monoculture. A ratio $r_{\text{bm}} > 1$ and a ratio $r_{\text{bm}} < 1$ indicate an increase and decrease in absolute abundance in the co-culture compared to monoculture, respectively. (B) Changes in the absolute abundance of a strain in co-culture compared to monoculture were determined and a pairwise interaction matrix was generated. Interactions where the individual abundance in co-culture significantly increased are indicated with (+), interactions where it significantly decreased are indicated with (–) and interaction where the abundance did not change in comparison to monoculture growth were indicated with (0). (C) Potentially cross-fed metabolites from C. innocuum I46 to E. faecalis KB1 were determined by comparing SM profiles (determined by untargeted MS) of KB1 and I46 for metabolites that are highly produced by I46 and consumed by KB1. Verified annotations are shown in green, potential annotations are shown in black and not annotated compounds are shown in grey as the corresponding feature identification numbers. (D) Time course of malate and L-methionine uptake by whole cells of E. faecalis KB1. Rates of $^{14}$C-malate uptake were measured at a final malate concentration of 10 µM at 18 °C. Standard deviations are shown from three biological replicates. (E) Using a serial passaging batch culture setup, the OMM\textsuperscript{12} community composition was analyzed after ten days of serial dilutions by comparing the relative strain abundances of ten replicates from two independent experiments in AF medium via qPCR. (F) Using the same approach, community composition of an OMM\textsuperscript{12}-KB1 dropout community was analyzed after ten days of serial dilutions by comparing the relative strain abundances of ten replicates from two independent experiments in AF medium.
Influence of specific supplements on community structure

Finally, we assessed the effect of media composition on community structure under our in vitro culture conditions. Therefore, we generated a comprehensive dataset comparing the composition of the complete OMM\textsuperscript{12} community and the E. faecalis KB1 dropout in media with different supplements that are known to promote the growth of specific gut bacteria but are missing in AF medium (mucin, C5/C6 sugars, xylan & inulin, starch; Figs. 5A, B, S19). We used a modified AF medium with reduced glucose concentration to rule out that substrate consumption may be inhibited by catabolite repression.
Of note, reduction of glucose resulted in decrease of E. faecalis KB1, C. innocuum I46, and B. animalis YL2 and increase of A. muciniphila YL44 (Figs. 5B, S19). We found that the chosen supplements had characteristic effects on relative and absolute abundance of individual strains (Figs. 5A, B, S19). A. muciniphila YL44, a known mucin-degrader, was boosted by xylan. Additional supplementation with C5/C6 sugars (xylose, arabino, lyxose, fucose and rhamnose) promoted growth of B. caecimuris I48. Further, supplementing media with xylan and inulin promoted growth of A. muris KB18 and even more enhanced levels of B. caecimuris I48. At the same time, A. muciniphila YL44 and M. intestinale YL27 were decreased. Interestingly, L. reuteri I49 was also promoted by xylan & inulin but only in the OMM11-E. faecalis KB1 dropout community (Figs. 5B, S19). Finally, supplementation of AF medium with starch only promoted growth of F. plautii YL31 (Figs. 5A, B; S19). Of note, increase of C. innocuum I46 and B. animalis YL2 strains was generally observed in the E. faecalis KB1 dropout communities irrespective of the media conditions. Lack of E. faecalis KB1 had different effects on the abundance of B. caecimuris I48, M. intestinale YL27 and F. plautii YL31 depending on supplements (Fig. 5A, B, S19).

**Comparison of in vitro and in vivo OMM12 community structure**

In order to evaluate, which of the in vitro conditions most closely resembled community composition in the murine gut, we analyzed community composition in the ileum, cecum, colon, and feces in 5 age-matched adult (12 weeks old) male C57Bl/6 mice. In general, intestinal communities were similar in cecum, colon, and feces, with B. caecimuris I48, B. cocoides YL58 and A. muciniphila YL44 predominating (Fig. 5C). The ileal community was distinct and dominated by B. cocoides YL58 and L. reuteri I49. The relative abundance of E. faecalis KB1 and F. plautii YL31 was comparatively low in in vivo samples. Similar as in AF media, A. muris KB18 and B. animalis YL2 were at the detection limit of the qPCR assay. Principal Component Analysis (PCA) of relative abundance data was used to compare in vivo community structures to OMM12 and OMM11-E. faecalis KB1 cultured in vitro in different media (Fig. SD). The highest similarity was observed between OMM12 community structure in cecum content and AF medium supplemented with mucin, C5/C6 sugars, xylan, and inulin. Conversely, OMM12 community structure in feces and colonic content most closely resembled OMM12 and/or OMM11-KB1 in medium M1 and M10. Taken together, we identified specific supplements that can be used to shape in vitro conditions to more closely recapitulate community structure in different regions of the murine gut.

**DISCUSSION**

A central challenge in gut microbiome research is to understand how interactions between the individual microorganisms affect community-level structure and related functions. Bottom-up approaches involving synthetic communities are valuable tools to study these interactions, as they allow to reduce complexity and to enable strain-specific manipulation. Using an in vitro approach, we focused on characteristics and interactions of the OMM12 community and combined monoculture, pairwise, and community cultivation of the strains with genome and metabonomics analysis of their spent media. Thereby we reveal that the OMM12 community interaction network is shaped by exploitative and interference competition in nutrient-rich culture media. In particular, E. faecalis KB1, a low-abundant member of the mammalian gut microbiota, was identified as an important driver of in vitro community composition by directly or indirectly altering the abundance of several other consortium members. We demonstrate that in vitro community structure can be modulated by specific supplements to more closely resemble the community in the murine gut. Additionally, we provide metabolic network reconstructions of the individual OMM12 strains, which are readily usable for in silico simulations and mechanistic studies using this synthetic community.

Exploitative (i.e., substrate) competition plays a major role in shaping intestinal bacterial communities [37]. Understanding the underlying principles of how bacteria compete for available nutrients is essential to predict and control community composition. We found that phylogenetically similar strains showed a higher substrate overlap (Fig. S8), which is in accordance with previous studies demonstrating that phylogeny reflects the metabolic capabilities of bacteria [38, 39]. Furthermore, overlap in substrate depletion profiles was correlated with growth inhibition in the respective SM (Fig. S9). This clearly indicates strong exploitative competition between individual OMM12 strains under the chosen in vitro conditions. We would like to note that the choice of culture media is an important deterministic factor of the observed competitive interactions and nutrient limitation may lead to more mutualistic interactions. B. caecimuris I48, E. faecalis KB1, E. clostridiformis YL32 and B. cocoides YL58 were found to consume a high number of substrates (>200), while their SM inhibited growth of the majority of the other community members including themselves (Fig. 1C). Of note, M. intestinale YL27 and C. innocuum I46 also consumed over 200 substrates each, but inhibited a few other strains. This demonstrates that substrate overlap cannot simply predict inhibition in all cases and other mechanisms (e.g., waste product inhibition) play a role in specific cases.

Besides substrate competition, a strain’s ability to acidify its environment or release an inhibitory factor (e.g., waste products, bacteriocins) can determine if another species can grow in the exhausted medium or not. Several strains, including B. caecimuris I48, B. cocoides YL58, E. faecalis KB1 and C. innocuum I46 acidified the medium during growth in monoculture. However, only for a few species, A. muciniphila YL44, B. caecimuris I48 and B. animalis YL2, acidic pH correlated with reduced growth (Fig. S4). Indeed, as reported previously, growth rate of these bacteria was strongly reduced at acidic pH [40] (Fig. S5). Moreover, the pH in the full OMM12 community in glucose rich AF medium, where most of the strains coexisted, was also slightly acidic (pH of 6.2), suggesting that pH modification does not play a major role in driving community composition under the conditions chosen. Interference competition by bacteriocins is widespread among gut bacterial communities [41]. We found that E. faecalis KB1 produces at least one antimicrobial compound that shows activity against...
five of the Gram-positive OMM12 strains (B. animalis YL2, E. clostridioformis YL32, F. plautii YL31, C. innocuum I46 and L. reuteri I49) (Fig. 1E). E. faecalis harbors genes coding for at least two different enterocins (enterocin LS0A/LS0B and enterocin O16). Therefore, we hypothesize that some of the inhibitory effects of E. faecalis KB1 on those strains can be attributed to enterocin-mediated killing. Generation of targeted deletions of the respective genes in E. faecalis will be required to prove that this mechanism indeed plays a role.

E. faecalis is a prevalent but low-abundant member of the undisturbed human and animal microbiota. Following antibiotic therapy, the bacterium can dominate the gut and cause bloodstream infection in immunocompromised individuals [42]. Understanding how E. faecalis out-competes/overgrows other gut microorganisms is important in order to intervene with E. faecalis domination in the gut. Besides antimicrobial-mediated inhibition of other bacteria our data suggest that metabolite cross-feeding contributes to the interaction of E. faecalis KB1 with C. innocuum I46 (Fig. 4A; B; Fig. S17). Based on metabolic profile mining we hypothesize that E. faecalis KB1 consumes malate, methionine, arginine and serine among other metabolites in co-culture with C. innocuum I46 (Fig. 4G). These metabolites may be secreted by C. innocuum I46 or alternatively, become available upon antimicrobial-mediated lysis. Interestingly, a previous study [43] reported that glucose-malate co-metabolism increases the growth of E. faecalis over glucose consumption alone. In connection with fast uptake rate of 14C-malate by E. faecalis KB1 (Fig. 4D), this suggests that malate cross-feeding may also contribute to E. faecalis KB1 gain in absolute abundance in co-culture with C. innocuum I46. In addition, E. faecalis KB1 may benefit from increased availability of shared substrates that become available upon enterocin-dependent inhibition. These mechanisms will have to be dissected in future work using E. faecalis KB1 mutants in enterocin production and metabolite uptake systems.

While dominating the in vitro community in glucose-rich complex media, the relative abundance of E. faecalis KB1 in different gut regions of adult mice was very low (<1%; Fig. 5). This raises the question whether in vitro observations can be translated to its role in the microbiota of adult mice. Encouraged by published work that reports high relative abundance of E. faecalis in newborn mice [44], we analyzed community composition in 7-day old OMM12 mice (Fig. S20). Of note, L. reuteri I49 and E. faecalis KB1 dominated the small intestinal community in these mice, suggesting that the environmental conditions in the neonatal gut foster growth of these Lactobacillales species. Most other OMM strains including B. animalis YL2 and F. plautii YL31, which are usually found in infant mice, were below the detection limit of our method. We conclude that the neonatal gut could be a highly relevant environment to study E. faecalis KB1 ecology due to its high abundance.

Using batch culture experiments we were able to investigate the assembly and dynamics of full OMM12 and OMM13-E. faecalis KB1 dropout communities in vitro. A significant increase in B. animalis YL2 and C. innocuum I46 in the community lacking E. faecalis KB1 suggested that enterocin-mediated killing and/or substrate competition could also shape the more complex community. Of note, the effect was absent in medium M8 containing additional C5/C6 sugars (Fig. S19). This suggests that both strains can co-exist when they do not have to compete for the same limiting substrate. Notably, in the full OMM12 community, ten of the twelve strains co-existed over ten days. This was remarkable given the high number of negative pairwise interactions in SM and co-culture experiments. Differences between the behavior of strains in pairwise versus complex communities point at higher-order ecological interactions that emerge in the community context. As previously shown in other studies, the underlying mechanisms may involve metabolic flexibility or mixed substrate utilization of the strains in the presence of competitors [45], metabolite cross-feeding, lack of waste-product inhibition, and overall change in pH [11] or an excess of provided substrates in the medium.

Following up, it will be important to assess if the in vitro findings can be translated to the mouse model. The choice of culture media that mimic in vivo substrate range and physiology is clearly a critical factor. It is well known that the environmental conditions vary along the length of the gastrointestinal tract. Accordingly, we also observed different OMM12 community in the ileum, cecum, colon and feces (Fig. 5C, D). For in vivo characterization, we have deliberately chosen AF media (M1), because it supports growth of all 12 OMM strains (Fig. S1) and similar media have also been used in previous studies ([46, 47]). Mucin, which is known to support growth of A. muciniphila and others [48, 49], has been explicitly omitted due to its side effects. First, it strongly increases culture turbidity which interferes with growth measurements. Moreover, commercially available mucin contains dead bacteria, which impedes routine sterility tests by microscopy and PCR. However, as addition of mucin to culture media clearly increases abundance of A. muciniphila YL44 in batch cultures, we recommend using it for future mechanistic in vitro studies of this model. Moreover, addition of C5/C6 sugars may also be considered as it boosts growth of B. cecimuris I48 and shapes in vitro community structure to more closely mimic structure in the murine cecum. Of note, F. plautii YL31, which shows low abundance in vivo, was strongly promoted by the addition of starch to the media (Fig. 5A), despite the lack of known pathways for starch utilization (Fig. 3A). We also found examples of functions predicted by the metabolic models (e.g., SCFA production) that could not be confirmed experimentally (Fig. 3A). This clearly shows that future work is needed to refine metabolic models with help of experimental data and identify and characterize hitherto unknown metabolic pathways. Apart from that, we have to study regulation of metabolic pathways under changing environmental in order to better predict the impact of environmental conditions on microbial community structure.

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

**METHODS**

**Generation of a 16S rRNA gene-based phylogenetic tree**

The genomes of the twelve strains of the OMM12 consortium [27] were accessed via DDBJ/ENA/GenBank using the following accession numbers: CP022712.1, NHM0R0200001-NHM0R0200002, CP021422.1, CP021421.1, NHMQ0100001-NHMQ0100005, NHTR0100001-NHTR01000016, CP021420.1, NHMP0100001-NHMP0100020, CP022722.1, NHMU0100001-NHMU0100019, NHMT0100001-NHMT0100003, CP022713.1 and annotated using Prokka (default settings) [50]. The 16S rRNA sequences of all strains were obtained. These rRNA FASTA sequences were uploaded to the SINA Aligner v1.2.11 [51] to align these sequences with a minimum 95%
Generation of genome-based metabolic models

Metabolic models were reconstructed using gapseq [1.1] with default settings [35]. In more detail, pathways were predicted as defined by the MetaCyc pathway database [36] and transporter were predicted based on the Transporter Classification Database (TCDB) [55] by using a bitscore cutoff of 200 for homology search. In agreement with experimental findings, the metabolic models were gap filled to allow in silico growth on AF medium which was prepared for computational applications accordingly [56]. The specific medium composition can be found in the supplementary file (AM_co.csv) and is encoded in the provided SBML models.

The individual components of the medium—and their respective quantities—were estimated based on the literature and assigned to the respective exchange reactions of the models. Shortly, the brain-heart infusion was inferred based on the composition of meat extract [57, 58]; the constituents of the trypticase soy broth can be found in the TSB medium in the gapseq repository [35]; the components of the yeast extract, it is published along with the above-mentioned protocol [56], and the carbohydrate and amino acid composition of fetal calf serum originates from the amino acid composition of selected Bos taurus proteins (NCBI Reference Sequence, https://www.ncbi.nlm.nih.gov/) along with textbook information [59]. Mucins were represented based on the respective mucus composition data as published previously [60].

A list of all MetaCyc pathways and their occurrence in the OMIM2 strains was pulled from the gapseq models and filtered to remove all pathways with an expected taxonomic range of eukaryotes only. Multiple pathways performing the same function were grouped according to the MetaCyc classification hierarchy, excluding compounded superpathways. The pathway groups shown in Figs. 3 and S10 as well as the transporters in Fig. S11 were chosen manually according to their interest for the manuscript and referring to the metabolic data.

Strains and culture conditions

Bacterial cultures were prepared from frozen monoculture stocks in a 10 ml culture and subculture in cell culture flask (flask T25, Sarstedt) previous to all experiments. Cultures were incubated at 37 °C without shaking under strictly anaerobic conditions (gas atmosphere 7% H2, 10% CO2, 83% N2). All experiments, except except experiments testing the influence of specific substrates on community composition, were carried out using AF medium (18 g/l brain-heart infusion (Oxoid), 15 g/l trypticase soy broth (Oxoid), 5 g/l yeast extract, 2.5 g/l KH2PO4, 1 mg/l haemin, 0.5 g/l D-glucose, 0.5 mg/l menadione, 3% heat-inactivated fetal calf serum, 0.25 g/l cysteine—HCl.H2O). For experiments testing the influence of specific substrates on community composition, AF medium was prepared with 15 g/l trypticase soy broth without added glucose (USBiological), as well as with the following substitute for brain-heart infusion: 6 g/l meat extract, 8 g/l nutrient broth (Difco) and 1.25 g/l Na2HP04. Using an enzyme-based colorimetric assay (Invitrogen, EIA/LGLC) glucose concentration in this glucose-reduced AF medium was determined as 0.6 g/l. Depending on the experimental condition mucin was added in a final concentration of 0.025%, fucose, xylose, arabinose, rhamnose and lyxose were added 0.5 g/l each, xylan from beechwood (Roth) and inulin from chicory extract (Roth) were added 2 g/l each and starch (Roth) was added 2 g/l.

For pH adjustment (Fig. S5), media was titrated with diluted NaOH and HCl.

The following strains were used in this study: Enterococcus faecalis KB1 (DSM32036), Bifidobacterium animalis YL2 (DSM26074), Acetilbacter mucis KB18 (DSM26090), Muribaculum intestinale YL27 (DSM28989), Flavonifractor plautii YL31 (DSM26117), Enterococcus clindamycinis YLS2 (DSM26114), Akkermansia muciniphila YL44 (DSM26127), Turicimonas muris YL45 (DSM26109), Clostridium innocuus I46 (DSM26113), Bacteroides thetaiotaomicron V7 (DSM26085), Limosilactobacillus reuteri I49 (DSM32035), Blautia cocoides YLS8 (DSM26115).

Growth measurements

Bacterial growth was measured in 96 well round bottom plates (Nunc) using a GenTech Epoch2 plate reader. Inocula were prepared from a previous culture and subculture and diluted in fresh AF medium at 0.01 OD600nm. Absorption at wavelength 600 nm was determined in a reaction volume of 100 µl in monoculture and SM experiments and 150 µl in co-culture experiments. During continuous measurements, the plate was heated inside the reader to 37 °C and a 30 s double orbital shaking step was performed prior to every measurement.

Generation of spent culture media

Bacterial cultures and subcultures were grown for 24 h each in 10 ml AF medium at 37 °C under anaerobic conditions without shaking. Bacterial spent culture supernatants (SM) were generated by centrifugation of the densely grown subculture at 4 °C for 20 min at 5000 x g and subsequent pH measurement and filter-sterilization (0.22 µm). SM samples were aliquoted and immediately frozen at −80 °C. Samples were thawed under anaerobic conditions previous to growth measurements. Growth of all bacterial monocultures in the spent culture media (SM) of all respective other strains was then measured as described above. SM were inoculated with bacterial monocultures with starting OD600nm 0.01. After monoculture growth of 20 h in the respective SM (resulting in double-spent media, DSM), pH values were determined.

pH measurements

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

Metabolic profiling of late stationary phase bacterial supernatants

The untargeted analysis was performed using a Nexera UHPLC system (Shimadzu) coupled to a Q-TOF mass spectrometer (TripleTOF 6600, AB Sciex). Separation of the spent media was performed using a UPLC BEH Amide 2.1 x 100, 1.7 µm analytic column (Waters Corp.) with 400 µl/min flow rate. The mobile phase was 5 mM ammonium acetate in water (eluent A) and 5 mM ammonium acetate in acetonitrile/water (95/5, v/v) (eluent B). The gradient profile was 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 12 to 15 min. A volume of 5 µl per sample was injected. The autosampler was cooled to 10 °C and the column oven heated to 40 °C. Every tenth run a quality control (QC) sample which was pooled from all samples was injected. The spent media samples were measured in a randomized order. The samples have been measured in Information Dependent Acquisition (IDA) mode. MS settings in the positive mode were as follows: Gas 1 55, Gas 2 65, Curtain gas 35, Temperature 500 °C, Ion Spray Voltage 5500, declustering potential 80. The mass range of the TOF MS and MS/MS scans were 50–2000 m/z and the collision energy was ramped from 15–55 V. MS settings in the negative mode were as follows: Gas 1 55, Gas 2 65, Curtain gas 35, Temperature 500 °C, Ion Spray Voltage −4500, declustering potential −80. The mass range of the TOF MS and MS/MS scans were 50–2000 m/z and the collision energy was ramped from −15–55 V. The “mconvert” from ProteoWizard [61] were used to convert raw files to mzXML (de-noised by centroid peaks). The biomicrodetector/R package xcms [62] was used for data processing and feature identification. More specifically, the matched filter algorithm was used to identify peaks (full width at half maximum set to 7.5 s). Then the peaks were grouped into features using the “peak density” method [62]. The area under the peaks was integrated to represent the abundance of features. The retention time was adjusted based on the peak groups presented in most of the samples. To annotate possible metabolites to identified features, the exact mass and MS2 fragmentation pattern of the measured features were compared to the records in HMDB [63] and the public MS/MS database in MSDIAL [64], referred to as MS1 and MS2 annotation, respectively. The QC samples were used to control and remove the potential batch effect, t-test was used to compare the features’ intensity from spent media with fresh media. The associated untargeted metabolomics data are available on Metabo-Lights repository [65] with ID MTLBS33553.

Targeted short chain fatty acid (SCFA) measurement

The 3-NPH method was used for the quantitation of SCFAs [66]. Briefly, 40 µl of the SM and 15 µl of isotopically labeled standards (ca 50 µM) were mixed with 20 µl 120 mM EDC HCl, 6% pyridine–solution and 20 µl of 200 mM 3-NPH HCL solution. After 30 min at 40 °C and shaking at 1000 rpm using an Eppendorf Thermomix (Eppendorf, Hamburg, Germany), 900 µl acetonitrile/water (50/50, v/v) was added. After centrifugation at 13,000 U/min for 2 min the clear supernatant was used for analysis. The same system
as described above was used. The electrospray voltage was set to –4500 V,
curtain gas to 35 psi, ion source gas 1 to 55, ion source gas 2 to 65, and
the temperature to 500 °C. The MRM-parameters were optimized using
commercially available standards for the SCFAs. The chromatographic
separation was performed on a 100 × 2.1 mm, 100 Å, 1.7 μm, Kinetex C18
column (Phenomenex, Aschaffenburg, Germany) column with 0.1% formic
acid (eluuent A) and 0.1% formic acid in acetonitrile (eluuent B) as elution
solvents. An injection volume of 1 μL and a flow rate of 0.4 mL/min was
used. The gradient elution started at 23% B which was held for 3 min,
afterward the concentration was increased to 30% B at 4 min, with another
increase to 40% B at 6.5 min, at 7 min 100% B was used which was held for
1 min, at 8.5 min the column was equilibrated at starting conditions. The
column oven was set to 40 °C and the autosampler to 15 °C. Data
acquisition and instrumental control were performed with Analyst
1.7 software (Sciex, Darmstadt, Germany).

Dynamic metabolic profiling of bacterial supernatants
All chemicals were purchased from Sigma Aldrich at the highest purity
available. 50 μl of the supernatants were spiked with 100 nmol sodium
pyruvate, 13C2 and 250 nmol norvaline as internal standards, afterwards
the samples were dried under a gentle stream of nitrogen. For derivatization
100 μl of a methoxyamine hydrochloride solution (10 mg/1 ml pyridine)
were added and the sample was shaken at 40 °C for 90 min. Afterwards
100 μl of MTBSTFA (N-(tert-butyldimethyl-silyl)-N-methyl-trifluoroacet-
amide containing 1% tert-butyldimethyl-silylchlorid) was added and the
sample was heated at 70 °C for 45 min. GC-MS-analysis was performed with a
QP2010 Plus or Ultra gas chromatograph/mass spectrometer (Shimadzu)
equipped with a fused silica capillary column (Equity TM-5: 30 m x 0.25
mm, 0.25 μm film thickness; SUPELCO) and a quadrupole detector working
with electron impact ionization at 70 eV. An aliquot of the derivatized
samples was injected in 1:5 split mode at an interface temperature of
260 °C and a helium inlet pressure of 70 kPa. After sample injection, the
column was first kept at 60 °C for 3 min and then developed with a
temperature gradient of 10 °C min–1 to a final temperature of 300 °C. This
temperature was held for further 3 min.

Pyruvate results were calculated relative to the pyruvate, 13C2 standard
(Rt 12.2 min), whereas all other metabolites were calculated relative to
norvaline (Rt 17.7 min).

For qualitative sugar analysis, 50 μl of the medium were dried under a
gentle stream of nitrogen. For derivatization 100 μl of a methoxamine
hydrochloride solution (10 mg/1 ml pyridine) were added and the sample
was shaken at 40 °C for 90 min. Afterwards 100 μl of MTBSTFA (N-methyl-
yl trifluoroacetamide containing 1% trimethylsilylchlorosilane) was
added and the sample was heated at 50 °C for 45 min. GC-MS-analysis
was performed as described above. Glucose, fructose, galactose, mannose
and trehalose were confirmed with standard solutions.

Spot assays
Bacterial cultures and subcultures were grown for 24 h each in 10 ml AF
medium at 37 °C under anaerobic conditions without shaking. Mono-
cultures were diluted to OD600nm 0.1 in a fresh AF medium. To generate
a dense bacterial lawn, monoculture inocula were diluted in LB soft agar to
OD600nm 0.01 and poured on a AF medium agar plate. After drying all
plate isolators (North Kent

Co-culture experiments
Monoculture inocula were prepared from a previous culture and subculture
and were diluted to OD600nm 0.1 in a fresh AF medium. Following, pairwise
cultures were generated by pooling diluted inocula in a 1:1 ratio. From each
culture 150 μl were set aside for qPCR measurements and determination of
initial relative abundances (timepoint 0 h). The remaining co-cultures were diluted
1:10 to OD600nm 0.01 and pipetted into a round-bottom 96-well plate (Nunc).
Growth measurements were performed as described above for 72 h. Samples
for qPCR analysis and pH measurements were taken every 24 h and the co-
cultures were serially diluted 1:100 into 150 μl fresh AF medium in a new 96-
well round-bottom plate to allow communities to approach a steady-state
composition over ~25 bacterial generations.

Animal experiments
C57Bl/6 mice stably associated with the OMM12 bacterial community were
housed under germ-free conditions in flexible film isolators (North Kent

Plastic Cages). Mice were supplied with autoclaved ddH2O and Mouse-
Breeding complete feed for mice (Sniff® ad libitum. For the experiment shown in Fig. 5C, 12 weeks old male mice were used and sacrificed by
cervical dislocation at ZG 10. Intestinal content was harvested, weighed,
and frozen at −20 °C before DNA extraction. For the experiment shown in Fig. 5D, 7 day old male and female mice were used and sacrificed by
decapitation at ZG 4. Intestinal sections were frozen at −20 °C before DNA
extraction.

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

DNA extraction from intestinal contents and in vitro community samples
gDNA extraction using a phenol-chloroform based protocol was performed as
described previously [25]. Fecal pellet or cecal content was resuspended in
500 μl extraction buffer (200 mM Tris-HCl, 200 mM NaCl, 20 mM EDTA in
ddH2O, pH 8, autoclaved), 210 μl 20 % SDS and 500 μl phenol:chloroform:
isoamylalcohol (25:24:1, pH 7.9). Furthermore, 0.1 mm-diameter zirconia/
silica beads (Roth) were added. Bacteria were lysed with a bead beater
(Tissuelyser LT, Qiagen) for 5 min. After centrifugation (14,000 xg, 5
min, RT), the aqueous phase was transferred into a new tube, 500 μl
phenolchloroform:isoamylalcohol (25:24:1, pH 7.9) were added and again
spun down. The resulting aqueous phase was gently mixed with 1 ml 96 %
ethanol and 50 μl of 3 M sodium acetate by inverting. After centrifugation
(30 min, 14,000 xg, 4 °C), the supranant was discarded and the gDNA
pellet was washed with 500 μl ice-cold 70 % ethanol and again centrifuged
(14,000 xg, 4 °C, 15 min). The resulting gDNA pellet was resuspended in
100 μl Tris-HCl pH 8. Subsequently, gDNA was purified using the the
Nucleospin gDNA clean-up kit (Macherey–Nagel) and stored at −20 °C.

Quantitative PCR of bacterial 16 S rRNA genes
Quantitative PCR was performed as described previously [23]. 5 ng gDNA
was used as a template for qPCR. Only for samples from infant mice
(Fig. 5C) 50 ng were used. Strain-specific 16S rRNA primers and hydrolysis
probes were used for amplification. Standard curves were determined using
linearized plasmids containing the 16S rRNA gene sequence of the
individual strains. The standard specific efficiency was then used for
absolute quantification of 16S rRNA gene copy numbers of individual
strains.

Determination of co-culture outcomes
Quantitative 16S rRNA copy numbers from the measurement endpoint
of three independent co-culture experiments were determined by qPCR. Co-
culture outcomes (positive, neutral, or negative) were determined by
calculating the individual abundance ratio for each strain in co-culture
relative to monoculture. Therefore, the strain-specific absolute abundance
at 72 h in all pairwise co-cultures was divided by the strain-specific
absolute abundance at 72 h in monoculture (Nstrain/NI) for every
individual experiment. Following, the mean abundance ratio from all
individual experiments (n = 3 per strain combination) was calculated.
Significance was determined using a two-sided t-test.

Community experiments
Monoculture inocula were prepared from a previous culture and subculture
and were diluted to OD600nm 0.1 in a fresh AF medium. Following, the community
inoculum with equivalent ratios of all 12 strains was

Malate and L-Methionine uptake measurements
Uptake of 14C-malate and 1H-L-methionine by E. faecalis KB1 was

10 mM MgCl$_2$ and resuspended in 50 mM Tris-maleate buffer (pH 7.2) containing 5 mM MgCl$_2$ thereby adjusting the cell suspension to an OD$_{600}$ of 10. For malate transport assays, this cell suspension was diluted 1:10 with 50 mM Tris-maleate buffer (pH 7.2) containing 5 mM MgCl$_2$. Uptake in 5 ml of scintillation fluid (MP Biomedicals), and radioactivity was determined in a liquid scintillation analyzer (PerkinElmer). Total protein content of E. faecalis cells in relation to OD$_{600}$ was determined with 20 µM carbonyl cyanide m-chlorophenylhydrazone (CCCP), 2 mM 2,4-dinitrophenol (DNP), 10 µM nonactin, 6 µM nigericin, 2 µM valinomycin or 20 µM MG132 (Biotrend), and with 5 mM MgCl$_2$, thereby adjusting the cell suspension to an OD$_{600}$ of 18. At various time intervals, the transport was terminated by the addition of stop buffer (100 mM potassium phosphate buffer, pH 6.0, 100 mM LiCl), followed by rapid filtration through membrane filters (MN gf-5 0.4 µm; Macherey-Nagel). The filters were dissolved in 5 ml of scintillation fluid (MP Biomedicals), and radioactivity was determined in a liquid scintillation analyzer (PerkinElmer).

The effects of protonophores and ionophores were tested after preincubation of cells in 50 mM potassium phosphate buffer (pH 7.2) containing 5 mM MgCl$_2$, and 1% peptone, supplemented with 20 µM carbonyl cyanide m-chlorophenylhydrazone (CCCP), and 2 mM 2,4-dinitrophenol (DNP), 10 µM nonactin, 6 µM nigericin, 2 µM valinomycin or dimethyl sulfoxide (DMSO), as a control at room temperature for 10 min.

Data analysis and figures

Data was analyzed using R Studio (Version 1.4.1103). Heatmaps were generated using the R pheatmap package (https://github.com/raivokolde/pheatmap). Plots were generated using the R ggplot2 package (69) and ggpubr package (https://github.com/kassambara/ggpubr). Figures were partly generated using BioRender (https://biorender.com) and Adobe Illustrator CC (Adobe Inc.).

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