Aus dem Max von Pettenkofer Institut
für Hygiene und Medizinische Mikrobiologie
Institut der Ludwig-Maximilians-Universität München
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Ecology of a Synthetic Gut Bacterial Community

Dissertation
zum Erwerb des Doktorgrades der Naturwissenschaften
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
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aus
Nürnberg, Deutschland

2022

Mit Genehmigung der Medizinischen Fakultät
der Universität München

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List of publications

Publications for the cumulative thesis:

1. **Weiss, A.S.**, Burrichter, A.G., Durai Raj, A.C., von Strempel, A., Meng, C., Kleigrewe, K., Münch, P.C., Rössler, L., Huber, C., Eisenreich, W., Jochum, L.M., Göing, S., Jung K., Lincetto, C., Hübner, J., Marinos, G., Zimmermann, J., Kaleta, C., Sanchez, A., Stecher, B. (2022)
   'In vitro interaction network of a synthetic gut bacterial community'
   The ISME Journal, 16: 1095–1109, doi.org/10.1038/s41396-021-01153-z

2. Eberl, C., **Weiss, A.S.**, Jochum, L.M., Durai Raj, A.C., Ring, D., Hussain, S., Herp, S., Meng C., Kleigrewe, K., Gigl, M., Basic, M., Stecher, B. (2021)
   'E. coli enhance colonization resistance against Salmonella Typhimurium by competing for galactitol, a context-dependent limiting carbon source'
   Cell Host Microbe, 29: 1-13., doi.org/10.1016/j.chom.2021.09.004

3. **Weiss, A.S.**, Niedermeier, L., Burrichter, A.G., von Strempel, A., Ring, D., Meng, C., Kleigrewe, K., Lincetto, C., Hübner, J., Stecher B. (2023)
   'Nutritional and host environments determine community ecology and keystone species in a synthetic gut bacterial community'
   Nature Communications, doi.org/10.1038/s41467-023-40372-0

Other publications not part of the cumulative thesis:

1. von Strempel, A., **Weiss, A.S.**, Wittman, J., Salvado Silva, M., Ring, D., Wortmann, E., Clavel, T., Débarbieux, L., Kleigrewe, K., Stecher, B. (2023)
   'Phage-targeting of protective commensals impairs resistance against Salmonella Typhimurium infection in gnotobiotic mice'
   PLOS Pathogens, doi.org/10.1371/journal.ppat.1011600

2. Plagge, J., Zimmermann-Kogadeeva, M., Höring, M., Slack, E., Heeren, J., Zimmermann, M., Giansanti, P., Basic, M., **Weiss, A.S.**, Seeliger, C., Bleich, A., Brunner, S., Hidrobo, M., Stecher, B., Coleman, O.I., Strohmeyer, A., Klingenspor, M., Küster, B., Janssen, K., Haller, D., Burkhardt, R., Kuipers, F., Liebisch, G., Ecker, J. (2022)
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4. Spriewald, S., Stadler, E., Hense, B.A., Münch, P.C., McHardy, A.C., **Weiss, A.S.**, Obeng, N., Müller, J., and Stecher, B. (2020)
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   mBio, 11(4), doi.org/10.1128/ mBio.00912-20

5. **Weiss A.S.**, Götz, A., Opitz, M. (2020)
   'Dynamics of ColicinE2 production and release determine the competitive success of a toxin-producing bacterial population'
   Scientific Reports, 10: 4052, doi.org/10.1038/s41598-020-61086-z

6. Götz, A., Mader, A., von Bronk, B., **Weiss, A.S.**, Opitz, M. (2020)
   'Gene expression noise in a complex artificial toxin expression system'
   PLOS ONE, 15(1): e0227249, doi.org/10.1371/journal.pone.0227249

Für Papa.

1 Abstract

The mammalian gastrointestinal tract harbours a variety of microbial organisms, together forming the gastrointestinal microbiota. The microbial community structure is shaped by several factors in the gut and its emerging functions have a decisive influence on a number of important health associated traits of its host. The role of the microbiome in health and different disease states has been investigated intensively. While several studies in the field of gut microbiome research claimed to have identified harmful or beneficial microbial species correlated with disease or host health, current findings indicate that in many cases microbiome function is rather determined by the microbial interaction network and its resulting function, than solely by the presence or absence of certain microbial organisms. Understanding microbial interaction patterns and the corresponding function of a complex microbial community therefore includes the description of metabolite dynamics influenced by the environmental system, the emerging metabolic processes on the single cell and population level and the resulting microbial ecology on the system's level.

To resolve the complexity of natural microbial ecosystems, the use of synthetic microbial communities such as the Oligo-Mouse-Microbiota (OMM^{12}) is a powerful tool for uncovering interrelationships between host and microbiome. Even though widely used, the interaction network of the OMM^{12} model remained largely understudied. Hence, a central aim of this thesis was to map out the metabolic capabilities and pairwise interaction patterns of the individual consortium members. Further, the presented studies provide a comprehensive reference dataset, as well as adaptable experimental protocols, enabling a more informed interpretation of future scientific work using this model community.

Making use of the gained insights allowed to use the established ecological tools to investigate the influence of the microbial community context on microbial functions, such as e.g. colonization resistance against invading enteric pathogens like *Salmonella enterica*. This revealed that in a gnotobiotic mouse model *Escherichia coli* in the context of the OMM^{12} community was able to protect the host from infection with *S. enterica* serovar Typhimurium (*S.* Tm) by more efficiently competing for a limiting nutrient. Systematically testing the metabolic capabilities of the community members elucidated the underlying mechanisms of niche exclusion, facilitated by the microbial community as a whole, but especially by the *Lachnospiraceae* strains of the consortium.

The important role of specific members of the consortium in emerging community functions like colonization resistance motivated the further investigation of the OMM^{12} model´s ecology and the role of individual species in community assembly and function. Therefore, in the third part of the thesis, we studied the community ecology of the consortium using a dropout community approach. Systematically removing single species from the community

and testing community assembly across different in vitro conditions and several regions of the murine gastrointestinal tract, revealed that the nutritional and host environment are key determinants of community structure. Further, individual community members were found to exert a particularly large influence on the abundance distribution of other species, depending on their capacity to manipulate the corresponding environment. Importantly, the study underlined the strong context dependency of bacterial interactions and a corresponding central ecological concept, the keystone species concept.
In conclusion, the presented findings help to create a fundamental understanding of microbial gut community ecology and the driving forces for community assembly, highlighting the important role of microbial ecology in gut health.

2 Zusammenfassung

Der menschliche Gastrointestinaltrakt beherbergt eine Vielzahl von mikrobiellen Organismen, die zusammen das Darmmikrobiom bilden. Die mikrobielle Gemeinschaft im Darm, deren Zusammensetzung und Struktur durch verschiedene Faktoren bestimmt wird, hat einen entscheidenden Einfluss auf die Gesundheit des menschlichen Wirts. Mehrere Studien auf dem Gebiet der Darmmikrobiomforschung versuchen, schädliche oder nützliche mikrobielle Spezies zu identifizieren, die mit bestimmten Krankheitsbildern des Wirts korreliert sind. Allerdings deuten aktuelle Erkenntnisse darauf hin, dass die Funktion des Mikrobioms in vielen Fällen eher durch das mikrobielle Interaktionsnetzwerk bestimmt wird, als allein durch die Anwesenheit oder Abwesenheit bestimmter mikrobieller Organismen. Zum Verständnis der mikrobiellen Interaktionsmuster und der entsprechenden Funktion einer komplexen mikrobiellen Gemeinschaft gehört daher sowohl die Beschreibung der vom Umweltsystem beeinflussten Metabolitendynamik, als auch der entstehenden Stoffwechselprozesse auf Einzelzell- und Populationsebene und der daraus resultierenden mikrobiellen Ökologie auf Systemebene. Um die Komplexität natürlicher mikrobieller Ökosysteme zu reduzieren, ist die Verwendung synthetischer mikrobieller Gemeinschaften wie der Oligo-Maus-Mikrobiota (OMM^{12}) eine hilfreiche Methode zur Erforschung von Wechselbeziehungen zwischen Wirt und Mikrobiom. Trotz der weiten Verbeitung des OMM^{12}-Modells, ist dessen Ökologie noch weitgehend unerforscht. Ein zentrales Ziel der vorliegenden Arbeit war es daher, die metabolischen Eigenschaften und paarweisen Interaktionsmuster der einzelnen Konsortiumsmitglieder zu charakterisieren und einen umfassenden Referenzdatensatz, sowie entsprechende Versuchsprotokolle bereitzustellen, die eine fundiertere Interpretation künftiger wissenschaftlicher Arbeiten mit dieser Modellgemeinschaft ermöglichen.

Die hier etablierten ökologischen Methoden wurden zudem genutzt, den Einfluss der mikrobiellen Gemeinschaft auf Funktionen des Darmmikrobioms zu untersuchen, wie z.B. die Kolonisierungsresistenz gegen Enteropathogene wie *Salmonella enterica*. Dabei zeigte sich, dass in einem gnotobiotischen Mausmodell *Escherichia coli* im Kontext des OMM^{12}-Modells in der Lage ist, den Wirt vor einer Infektion mit *S. enterica* serovar Typhimurium (*S.* Tm) zu schützen, indem das Bakterium effizienter um einen limitierenden Nährstoff konkurriert. Durch die systematische Untersuchung der metabolischen Charakteristika der Mitglieder der Gemeinschaft, wurden die zugrundeliegenden Mechanismen aufgeklärt, in denen die mikrobielle Gemeinschaft als Ganzes, insbesondere aber die *Lachnospiraceae*-Stämme des Konsortiums, eine wichtige Rolle spielen.

Die Erkenntnis, dass spezifische Mitglieder des Konsortiums entscheidende Mikrobiomfunktionen stark beeinflussen, motivierte die weitere Untersuchung der Ökologie des OMM^{12}-Modells. Daher wurde im dritten Teil der Arbeit die Gemeinschaftsökologie des Konsor-

tiums untersucht. Das systematische Ausschließen einzelner Arten aus dem Konsortium und Analysieren der sich ergebenden Strukturbildung unter verschiedenen in vitro Bedingungen und in verschiedenen Regionen des Gastrointestinaltrakts von Mäusen ergab, dass die biotische und abiotische Umgebung die Ökologie der Gemeinschaft maßgeblich bestimmen. Darüber hinaus wurde festgestellt, dass einzelne Mitglieder der Gemeinschaft durch ihre Fähigkeit, die entsprechende Umgebung biochemisch zu manipulieren, einen besonders großen Einfluss auf die Abundanz anderer Arten ausüben. Die Studie unterstreicht demnach die starke Kontextabhängigkeit bakterieller Interaktionen und die damit eines zentralen ökologischen Konzepts, des "Keystone Species" Konzepts.
Zusammenfassend tragen die vorgestellten Ergebnisse zu einem grundlegenden Verständnis der Ökologie mikrobieller Darmgemeinschaften bei und unterstreichen die wichtige Rolle der mikrobiellen Ökologie für die Darmgesundheit.

3 Introduction

3.1 The gastrointestinal microbiome

Microbial communities of bacteria and other microorganisms are found in all ecosystems of our planet, from the oceans, to the rhizosphere, to the skin and intestines of most animals, including humans. Especially the mammalian gastrointestinal tract hosts a variety of microorganisms, including bacteria, fungi, protists and viruses, all interacting with each other and the host system, by that forming the so called gastrointestinal microbiota [1, 2]. With an abundance of roughly 10^{14} microorganisms, bacteria make up a substantial part of this ecosystem [3]. A balanced and stable coexistence of a huge variety of bacterial populations was shown to be crucial to the well-being of the host living in symbiosis with its prokaryotic residents [4, 5]. As next generation sequencing enabled scientists to investigate the correlation between the microbial composition and human diseases [6], it has been shown that many gastrointestinal and metabolic diseases, as for example inflammatory bowel disease [7] or diabetes type 2 [8], are linked to a shift in the composition of the gastrointestinal microbiome.

3.1.1 The role of microbial ecology in gut health

Due to the apparent connection between the gut microbiome and human health, it is crucial to understand what determines the composition of microbial communities, how biodiversity is maintained and which deterministic and stochastic processes affect microbial population dynamics in the gastrointestinal system. While many studies focus on taxonomic analysis of disease correlated gut microbiome composition, it has become clear that microbiomes are highly complex and dynamic systems, that are not only shaped by their phylogenetic structure, but by the various and adaptive interactions in between the microbes themselves and with their environment [9, 10]. Recent work suggests that phylogenetic identity and microbial ecology together distinctly shape manifold microbiome functions [11]. Therefore, to fully understand what characterizes a health-associated microbiome and which changes occur when substantial perturbations develop within the host system, mechanistic analysis of the microbiome functionality via its metabolome and metagenome, but also the resulting interaction mechanisms and dynamics are needed. Hence, a major challenge of microbiome research is to disentangle several interdependent entities: a) the fundamental microbial ecology, b) the resulting microbiome functions and c) the corresponding host interactions (Fig. 3.1). A complex example of an important function of the gut microbiota that requires an understanding of all three factors, is the protection against invading enteric pathogens, called colonization resistance (CR). Employing a combination of different key mechanisms like

resource competition, direct inhibition of competitors and host immune response stimulation, complex gut microbial communities successfully hinder infection with enteropathogens [12]. Several studies confirm the importance of mechanisms well-known from microbial ecology, that enable the resident microbiota to provide CR. Prominent examples include the competition for resources of commensal *Enterobacteriaceae* with invading *Salmonella* serovars for oxygen [13], iron [14] or mucosal sugars [15]. Another CR relevant mechanism of action is the direct inhibition of invading pathogens. For example it was shown, that accumulating SCFAs like propionate, produced by *Bacteroidetes* species, strongly attenuate growth and virulence of *Salmonella enterica* and by that inhibit proliferation of the pathogen in the gut [16]. Not only metabolic by- or end-products can serve as inhibiting agent, but several bacteria are known to produce antimicrobial compounds that enable the targeted inhibition or killing of competitors [17]. Such so called bacterial toxins, or bacteriocins, are e.g. produced by the probiotic *E. coli* Nissle in the inflamed gut that negatively affect its competing target *S. enterica* [18].

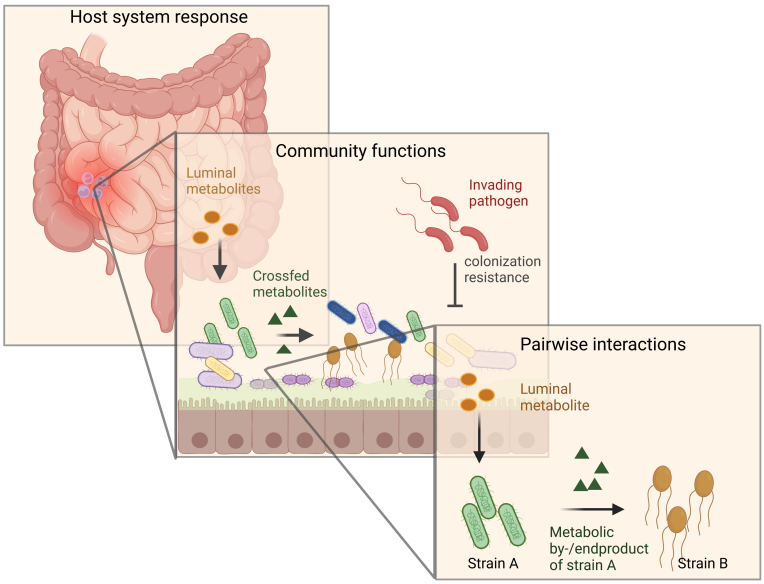

Figure 3.1: Interdependent entities together facilitating complex functions like colonization resistance (CR): a) the fundamental microbial ecology, as depicted by pairwise bacterial interactions, b) the emerging microbial community behaviour and c) the corresponding host system response, as e.g. immune reaction. (Figure created with BioRender.)

Concepts of microbial ecology further find application in the context of gut health when studying the stability of gut microbial communities. Numerous studies have shown, that a perturbation of the microbiome is often strongly linked to the disruption of global functions of the host's metabolism and immune system [19]. While the human gastrointestinal microbiome is remarkable in its intrinsic stability and resilience [20], strong perturbations as antibiotic treatments, infections or systemic diseases can lead to a loss of biodiversity or drastic shifts in microbiome composition [21]. Hence, ecological concepts can help to investigate what

causes stability and resilience of microbial communities in response to perturbations and which factors may drive the recovery of a perturbed system.

3.1.2 Challenges in gut microbiome research

Technological advances in bioinformatics and 'omics'-approaches provided a more comprehensive view on the structure and functional potential of the gut microbiome across individuals [22, 23]. Nevertheless, computational efforts to process these diverse sets of microbiome analyses still fail in reliably predicting generalizable features of health associated microbial communities [24]. This points towards a gap in knowledge when it comes to translating experimental readouts to microbiome function. This is likely due to the overwhelming complexity of the underlying mechanisms and the taxonomic diversity of the intestinal microbiome. Especially in individuals, tremendous differences in microbiome composition are observed on the strain level, which can be partly explained by demography, ethnicity, age, health status, drug intake and dietary habits [25]. The accessibility of 16S ribosomal RNA (rRNA) gene sequences underlined the taxonomic diversity of gut bacterial communities. But several relevant ecological functions are known to be encoded in the accessory genome or are encountered by specific co-evolution of microbes with their host, challenging descriptive theories in combining the richness and potential of bacterial genomes with bacterial community ecology [26, 11]. Some ecological traits can even be exchanged among members of a community, e.g. plasmids or mobile genetic elements [27], but are not found in the same species inhabiting a different niche. While several studies in the field of gut microbiome research claim to have identified harmful or beneficial bacterial species correlated with disease or host health, current findings indicate that in many cases microbiome function is rather determined by the bacterial interaction network and its resulting metabolic function, than solely by the presence or absence of certain bacterial species [9]. Understanding bacterial interaction patterns and the resulting effects on microbiome function of a complex bacterial community therefore includes the description of metabolite dynamics influenced by the environmental system, the emerging metabolic processes on the single cell and population level and the resulting microbial ecology on the system's level. In short, to truly understand the role and function of a microbial community member it has to be investigated in the relevant context, including the other members of its community.

3.2 Approaches in gut microbial ecology

Motivated by the wide-ranging effects of gut microbial ecology in host health, gut microbiome research aims to decipher microbiome signatures, specific markers of microbial communities and their functions that causally relate to specific host phenotypes. To disentangle the complexity of the trophic networks that evolved in the mammalian intestine, different approaches have been chosen. In "top-down" approaches studies work directly with highly complex data sets, often generated using 'omics'-technologies. This includes metagenomics, metatranscriptomics, metaproteomics and metabolomics, all methods that produce an incredible richness of data, which can be exploited by modelling approaches to

shed light on the underlying processes shaping the microbiome [23]. In particular, inference of microbial interaction by e.g. correlation algorithms and co-occurrence networks have proven helpful to delineate microbial community structures and to decipher the observed complex microbial patterns from microbiome profiling data [28]. Though, while certain functions or species can be correlated with disease patterns or other phenotypes, causality often cannot necessarily be inferred as the biological interpretation often remains uncertain and would require experimental validation [26].

3.2.1 Synthetic bacterial communities

One way to allow for specific correlation of observed phenotypes and hypothesis driven experiments is to work with synthetic microbial consortia in highly controlled environmental conditions. Synthetic microbial communities, meaning the controlled composition of selected microorganisms, can be constructed using different approaches [29]. To construct communities that can be used to recapitulate specific microbiome signatures related to an observed phenotype [30], community members can be selected based on genomic profiles of the taxonomic diversity of an acquired sample. This approach requires the possibility to isolate the selected bacteria, phages or other microorganisms from a given sample [31]. Especially the lack of specific selection factors in cultivation media and oftentimes particular biotic or abiotic requirements of some microorganisms, depending on the origin of the sample, pose a bottleneck in the assembly of synthetic communities from environmental samples. Another strategy combines individual microorganisms that were previously isolated and are well characterized. Individual microbial species with particular features can be combined to assemble a synthetic community in a hypothesis driven way [32]. While the use of previously cultivated and studied organisms provides a strong advantage in handling and designing experimental setups, such communities rarely reflect the signatures of natural systems and are strongly biased in their metabolic capabilities as they are selected to be growing in a given laboratory environment.

A pioneer in the field of synthetic gut bacterial communities was Russel W. Schaedler, who established a defined mixture of six cultivable bacterial strains isolated from mice already in 1965 [33]. The aim of this early work was to colonize laboratory mice with a microbiota free of known mouse pathogens. This minimal consortium was later modified to contain eight obligate anaerobic species that represent several major eubacterial constituents of the mouse gastrointestinal bacteria [34] and the model is used in different modifications still today in in vitro approaches, as well as in gnotobiotic mouse models [35, 36]. While the design of this synthetic community marked an important step in gut microbiome research, the ASF fails to recapitulate many major functions of the enteric microbiota in gnotobiotic mice, such as colonization resistance against invading pathogenic organisms [37]. Further, the selected strains are not available in public strain collections, complicating the modification of the consortium and replicability of observed phenotypes. Therefore, other more comprehensive collections of mouse gut bacteria were established and made available to the scientific community, as e.g. the mouse intestinal bacterial collection (miBC) [38]. This publicly available collection of bacteria was recently expanded and contains now 212 strains,

including a substantial amount of novel taxa [39].

3.2.2 The Oligo-Mouse-Microbiota

Another example of a mouse-derived synthetic community is the Oligo-Mouse-Microbiota (OMM^{12}) [32]. This synthetic gut bacterial community consists of twelve bacterial strains isolated from the intestine of mice that represent five abundant phyla of the mouse microbiome (Bacillota, Bacteroidota, Verrucomicrobia, Actinomycetota and Pseudomonadota) [40].

Figure 3.2: Phylogenetic tree of the Oligo-Mouse-Microbiota (OMM^{12}) based on 16S rRNA gene seqeucens, adopted from Weiss et al. [41].

The consortium was developed with a clear focus to disentangle host–microbe and microbe–microbe interactions and to resolve the contribution of individual bacteria to metabolic and host-related phenotypes. The individual strains have been deposited at public strain collections, are available for non-commercial use and are fully genome-sequenced [42, 43]. Further, a main goal was to develop a synthetic consortium that could be used in a controllable and reproducible manner in different laboratories, while still reproducing physiologically relevant parameters of the complex natural microbiota. To this end, more than 60 strains were initially isolated, sequenced and phylogenetically characterized. From this database, strains were then selected that met the following criteria: reproducible and reliable cultivation in vitro, stable cryo-preservation, and sufficient representation of the genetic diversity of the natural system. Finally, the OMM^{12} community includes the following twelve strains: *Enterococcus faecalis* KB1, *Limosilactobacillus reuteri* I49, *Clostridium innocuum* I46, *Bifidobacterium animalis* YL2, *Blautia coccoides* YL58, *Enterocloster clostridioformis* YL32,

Flavonifractor plautii YL31, *Acutalibacter muris* KB18, *Muribaculum intestinale* YL27, *Bacteroides caecimuris* I48, *Akkermansia muciniphila* YL44 and *Turicimonas muris* YL45 (Fig. 3.2). A 16S rRNA-based qPCR [32] and FISH [44] probes allow for quantitative tracking of the community members, as well as for spatially resolved imaging. Due to its high practicability, the OMM^{12} model has by now been used in more than 50 different laboratories worldwide and is characterized by its versatility in the field of gut microbiome research. Thus, it finds application in various research areas, ranging from research questions on immunology [45], metabolic diseases [46] and in infection biology [47, 48].

3.3 Principles of microbial ecology

The characteristics of bacterial communities is strongly influenced by deterministic interactions between the individual species and their corresponding environment [49]. Across ecosystems, and especially in the mammalian gut, microbial communities often face frequent changes in the availability of nutrients, as the sources of metabolites vary in space and time in dynamic conditions [50, 51]. As a consequence, complex interaction networks especially between bacterial species have evolved, increasing the robustness and distinctly shaping the community function of microbial communities [52]. Therefore, to eventually understand and control gut microbial communities, a central aim of microbiome research is to study bacterial interaction patterns, community ecology and the interdependency between community characteristics and the corresponding nutritional and chemical environment [11].

3.3.1 Bacterial interaction patterns

Bacterial ecology classifies inter- or intraspecies interaction patterns as antagonistic, neutral or beneficial (Fig. 3.3). While antagonistic interactions include exploitative competition for the same nutritional substrates [53] and interference competition e.g. via bacterial toxins [54] (Fig. 3.3A), beneficial or mutualistic interactions include syntrophy and cross-feeding [55] (Fig. 3.3C).

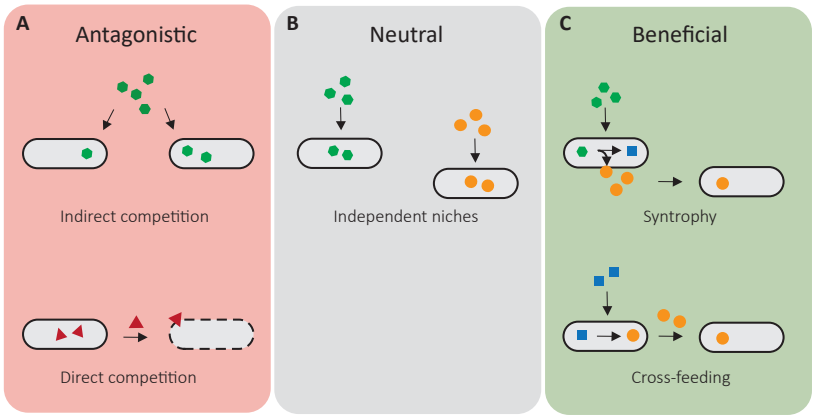

Figure 3.3: Bacterial interaction patterns include antagonistic (A), neutral (B) and beneficial (C) interaction types. (Figure created with BioRender.)

Syntrophic interactions describe the consumption of an intermediate or end metabolite from one organism by another that facilitates an otherwise energetically unfavorable reaction [56]. A typical example for syntrophy is interspecies hydrogen transfer in the ruminant's digestive system [57]. Here, hydrogen consuming organisms like sufate-reducing bacteria and acetogens utilize the hydrogen produced by the anaerobic fermentation of organic matter to short-chain fatty acids (SCFAs) by coexisting species [58]. The active consumption of hydrogen in turn allows secondary fermentation of products such as propionate to become energetically favorable. Cross-feeding on the other hand implies the production of a central nutrient by one organism that benefits the growth of another organism [57, 56]. An example for cross-feeding in the gastrointestinal system is the production of riboflavin by *Lactobacillus paracasei*, which is essential for the growth of *Faecalibacterium prausnitzii* [59]. Both, syntrophy and cross-feeding can be uni- or bidirectional, meaning that beneficial interaction types are not only mutualistic, but can find their origin in more complex ecological relationships. Therefore, metabolic interactions can be categorized by the following aspects: (i) the investment by the involved partners (syntrophic byproduct or cooperative cross-feeding), and (ii) the degree of reciprocity (uni- or bidirectional) [60]. Independent coexistence of two organisms due to the consumption of different metabolic substrates can be described as the occupation of a specific metabolic niche (Fig. 3.3B). More quantitatively, bacterial interaction networks can be described based on the net fitness effects that result for the organisms involved, meaning that pairwise interactions can have three possible outcomes: negative (-), neutral (0) or positive (+) [61].

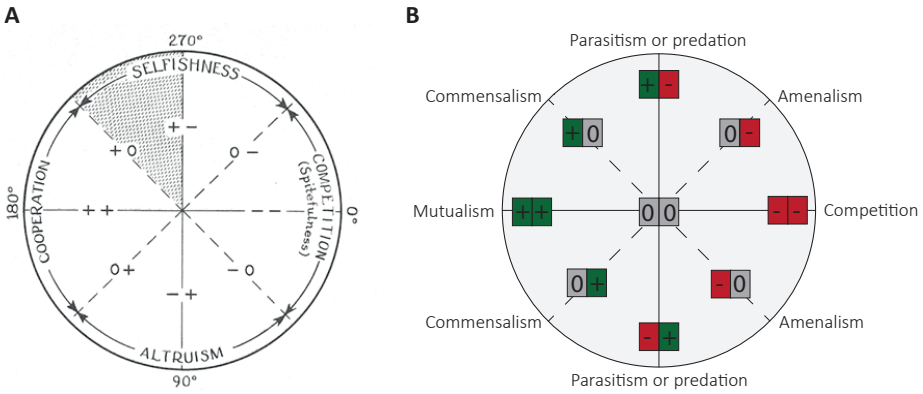

Figure 3.4: "Intra-Action Compass" designed by Lidicker describing the interactions occurring among members of the same or different species in the original version (A) [62] and the adapted version (B) [61].

Based on this assumption, William Lidicker designed the "Intra-Action Compass" in 1979 [62] describing the possible interaction types occurring among organisms (Fig. 3.4A). Here, each quadrant represents the quality of a specific interaction with the effect on one organism held constant, while the other is varied from strongly positive to strongly negative. Lidicker's original scheme results in four interaction types: altruism, cooperation, selfishness and competition. In (Fig. 3.4B) an adapted version of Lidicker's "Intra-Action Compass" is shown,

summarizing all possible interaction types [61].

3.3.2 Bacterial community networks

Bacterial species rarely occur in isolation, but live in multi-species communities. Many microbial ecosystems stand out with their diversity and richness in taxonomic membership [63]. Therefore, interactions among the individual community members are not limited to pairs, but can occur in groups of larger size. Despite only a limited number of bacteria can encounter in interactions at a given time, the outcomes of these interchanges can have an incremental impact that quickly multiplies in the resulting effects, by that affecting the macroscopic behavior of a system, as e.g. the resilience of the microbial community upon perturbation [64]. As bacteria simultaneously interact on the microscopic level, interaction patterns of multi-species communities become non-linear [65, 66]. Interactions of more than three individuals are therefore called higher-order interactions and describe for instance, how a third species can alter the interaction between two other species (Fig. 3.5). Consequently, the functionality of a community can develop to more than what would be expected from the individually observed phenotypes and can often not be predicted solely by studying the behavior of single organisms or pairs. This observation is called emergent behavior [67]. A complex example of emergent behavior of the gut microbiota is the protection against invading enteric pathogens, called colonization resistance (CR) (see section 3.1.1).

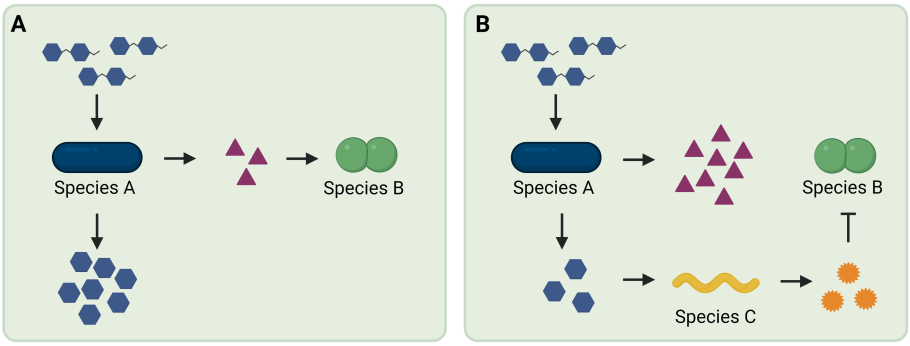

Figure 3.5: Exemplary outline of changes in interaction patterns in a multispecies community. A beneficial cross-feeding interaction between a given species A and species B (**A**) is altered by the presence of third species C (**B**). By producing an inhibiting compound species C is negatively affecting species B, by that changing strain relationships between the initial strains and metabolic fluxes of the community. (Figure created with BioRender.)

Identifying true higher-order interactions would require to investigate all possible interaction outcomes in a given community. Experimentally testing all possible interactions is not feasible for most bacterial communities of interest. Therefore, bacterial ecology relies mainly on computational approaches, or indirect and combinatorial experimental setups.
To decipher complex microbial interaction patterns, network-based approaches are often used to infer microbial ecology from microbiome profiling data [68]. Several studies using

metabolomics, transcriptomics and co-occurrence analyses from diverse datasets provided insights into functioning and dynamics of microbial communities [28]. Such approaches often lack traceability of individual community members and experimental means to resolve interaction networks and biochemical mechanisms. Further, the computationally identified bacterial associations might result from true ecological interactions between microorganisms, but cannot be distinguished from associations occurring due to environmental selection [24]. To causally link bacterial interactions to community functions, synthetic bacterial communities are a helpful reductionist tool (i.e. communities assembled from cultured representatives, see section 3.2.1). Using synthetic communities facilitates combinatorial approaches like the generation of drop-out or drop-in communities [69, 35]. Here, the systematic removal or addition of individual community members allows to compare community assembly or function across experimental environments [70, 71]. Combining insights with data from strain behavior in monocultures or pairwise co-cultures can provide insights into the ecology of a community and the corresponding interaction network, without testing all possible interaction configurations.

3.3.3 The keystone species concept

As bacteria are impressively versatile and adaptable, across complex environments most members of a community can establish different types of direct or indirect interactions with other species in their surroundings, thus potentially playing multiple ecological roles in a given ecosystem. Despite the potential diversity, across biological ecosystems, often single or a small number of species of a given community are found to play an especially important role. As the first to describe this observation, Robert T. Paine formulated the keystone species concept in 1969, based on experiments with starfish in tidal ponds [72]. Studying the influence of the starfish *Pisaster ochraceus* on the intertidal ecosystem, he came to the conclusion that distribution and density patterns of occuring species can be disproportionately affected by the activities of a single species, in this case *P. ochraceus*, of high trophic status [72] - later termed keystone species. This concept was successfully adapted to all sorts of ecological systems, including microbial ecology. A common definition characterizes bacterial keystone species as community members that disproportionately affect ecological processes, by that playing a more important role than other (often more abundant) members of the community [73, 74]. Even though, both abundance and richness of species were found to have strong effects on ecosystem dynamics where abundant community members are frequently found to be important for community characteristics [75, 76], species functionality is equally important in determining keystone members. A species' functional importance can be described as the result of two factors, that determine the effectiveness of interactions [77, 78]: a) the quantitative component which is driven by the abundance of a given species, determining the probability to encounter and affect other species of the community and b) the qualitative component which is determined by the physiological and ecological traits of the interacting species [79]. To comprehensively describe the role of keystone species in a community of interest therefore requires to study these components independently [74].

3.4 Aims of this thesis

3.4.1 Interaction network of a synthetic gut bacterial community

As outlined, the understanding of microbial ecology in the context of the gastrointestinal ecosystem (see section 3.1.1) is crucial in order to be able to identify and maintain a healthy microbiome or to engineer it in a way that utilizes its therapeutic potential. The Oligo-Mouse-Microbiota (see section 3.2.2) is a synthetic gut bacterial community model and even though it is widely used in several mouse models for human diseases, very little was known about the characteristics of the individual community members and their ecological relationships. Therefore, one goal of this doctoral thesis was to study the growth characteristics and metabolic potential, as well as the interactions between the members of the OMM^{12} community in vitro. Studying pairwise interactions (as described in section. 3.3.1) of all strains revealed that *E. faecalis* KB1 and *B. coccoides* YL58 are dominant drivers of interactions in vitro and that the pairwise interaction network is shaped by exploitative as well as interference competition in a glucose-rich culture medium [41]. Moreover, the study explored the metabolic capabilities of the individual strains, generated genome-based metabolic network reconstructions and identified the main producers of short chain fatty acids and amino acids. In summary, the first study presented in this doctoral thesis provides a comprehensive reference dataset, as well as adaptable experimental protocols, enabling a more informed interpretation of future scientific work using the OMM^{12} model.

3.4.2 Influence of the microbiome context on bacterial functions

Bacterial interaction patterns and the resulting effects on microbiome function are tightly connected to the corresponding environmental system. While complex microbial community functions like colonization resistance (see section. 3.1.1) are shaped by distinct pairwise interactions between specific bacterial species, the surrounding or "background" microbial community can strongly influence the interaction network (see section. 3.3.2). This motivated the investigation of the influence of the bacterial community context on bacterial functions. The second study presented in this thesis focuses on the protective function of *Escherichia coli* against invading *Salmonella enterica* serovar Typhimurium (*S.* Tm) in the murine gastrointestinal tract [48]. Systematically testing the influence of *E. coli* on *S.* Tm loads across different microbiota contexts, revealed that only in the context of the OMM^{12} community, but not the ASF community, *E. coli* can protect the host against infection. Depending on the background microbiota, *E. coli* and *S.* Tm could either directly compete with each other or coexist in the gut. Coexistence of two organisms due to the consumption of different substrates can be described as the occupation of a specific metabolic niche (Fig. 3.3B). Ecologically this behavior can be explained by Freter's nutrient niche theory [80], stating that an organism can only colonize a system if it is able to utilize one or a few limiting nutrients more efficiently than its competitors. Hence, in the context of the OMM^{12} community *E. coli* was able to close the niche available for *S.* Tm by more efficiently using the limiting nutrient. Using approaches from bacterial ecology, we aimed to elucidate the

underlying mechanisms of niche exclusion by exploring the metabolic capabilities and niche overlap of the members of the OMM community and the target strains *E. coli* and *S.* Tm.

3.4.3 Influence of the biotic and abiotic environment on bacterial ecology

The important role of specific members of the consortium in emerging community functions like colonization resistance, motivated the further investigation of the OMM^{12} model´s ecology and the role of individual species in community assembly and function. As described above (section. 3.3.3), the systematic experimental identification and description of keystone species in a given microbial community requires to account for both, the species abundance and the physiological and ecological traits of the interacting species. The myriad of potential interaction mechanisms and ecological functions in complex bacterial communities implies that keystone species might not be equally important across multiple metabolic environments. Hence, when setting out to explore the community ecology of the OMM^{12} consortium, we aimed to analyse the bacterial interactions across different nutritional conditions. Therefore, the third study presented in this doctoral thesis investigated community assembly and strain relationships of dropout communities and the full community in an in vitro batch culture approach in different commonly used anaerobic cultivation media, as well as across the different regions of the murine gastrointestinal tract [81]. These systematic analyses revealed strong dependency of keystone functions and community ecology on the environmental context. In summary, the findings of this study urge the need for a concrete specification of the keystone species concept and underline the need for approaches making use of controllable community models, traceable nutritional environments and a combination of metagenomics and metabolomics approaches.

4 Bibliography

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5 Publications

5.1 Publication I

5.1.1 Summary and contributions to publication I

In the manuscript "In vitro interaction network of a synthetic gut microbial community" published in the ISME Journal 2022, (Authors: Weiss, A.S., Burrichter, A.G., Durai Raj, A.C., von Strempel, A., Meng, C., Kleigrewe, K., Münch, P.C., Rössler, L., Huber, C., Eisenreich, W., Jochum, L.M., Göing, S., Jung, K., Lincetto, C., Hübner, J., Marinos, G., Zimmermann, J., Kaleta, C., Sanchez, A., Stecher, B.), we extensively characterized the individual members and performed a comprehensive analysis of the interaction network of the OMM^{12} synthetic gut bacterial community. The OMM^{12} is a community model frequently used in gut microbiome research. The generated data base published in this study is the first in vitro description of the metabolic and ecological characteristics of this model community and thereby provides a valuable tool for researchers working with this model system across different research areas. Using a bottom-up approach, we systematically identified the directionality of strain-strain interactions in spent media and pairwise co-culture experiments and developed a community batch culture model. Genome-informed metabolic network reconstruction in combination with targeted and untargeted metabolomics analysis of bacterial culture supernatants provided insights into the fundamental and realized metabolic potential of the individual community members. Doing so, we could show that the OMM^{12} interaction network is shaped by exploitative and interference competition and demonstrate how community composition can be shifted by changing the nutritional environment in vitro. I am the sole first author of this study. The study was designed by me and my supervisor Bärbel Stecher. I performed growth characteristics of the individual strains, spent media experiments, preparation of samples sent for untargeted and targeted metabolomics analyses, pH profiling, phenotyping of enterocin production in *Enterococcus faecalis*, curation of genome-based metabolic models, as well as co-culture experiments. I developed a community batch culture model, conducted community experiments across different nutritional conditions in vitro and performed mouse experiments determining community compositing in vivo across different sampling sites of the murine gut, as well as community assembly across the gastrointestinal tract in infant mice. I performed the analysis of data sets provided by collaborators Durai Raj, Meng and Kleigrewe, Rössler, Huber and Eisenreich, Lincetto and Hübner, Marinos, Zimmermann and Kaleta and generated all figures except Fig. 1E (spot assays) and Fig. 4D (transport assays). The draft manuscript was written by me, Lara M. Jochum and Bärbel Stecher. All authors contributed in reviewing and editing the draft manuscript.

5.1.2 Manuscript I

The manuscript is available here: https://doi.org/10.1038/s41396-021-01153-z

5.2 Publication II

5.2.1 Summary and contributions to publication II

In the manuscript "*E. coli* enhance colonization resistance against *Salmonella* Typhimurium by competing for galactitol, a context-dependent limiting carbon" published in Cell, Host Microbe 2021, (Authors: Eberl, C., Weiss, A.S., Jochum, L.M., Durai Raj, A.C., Ring, D., Hussain, S., Meng, C., Kleigrewe, K., Gigl, M., Basic, M., Stecher, B.), we describe new insights into *E. coli* mediated colonization resistance against *Salmonella enterica* serovar Typhimurium (*S.* Tm) infections using synthetic bacterial communities. Using a combination of phenotyping approaches, gnotobiotic mouse models, transcriptomics and metabolomics the study uncovered the crucial role of the microbiota context in how *E. coli* prevents *S.* Tm ecosystem invasion. Only in the presence of other sugar-consuming members of our synthetic bacterial community, was *E. coli* found to deplete galactitol, a diet-derived sugar alcohol, thereby together with the microbiota establishing niche exclusion of invading *S.* Tm. This work underlines the importance of the microbial context in finding mechanistic understanding of bacterial community functions, as colonization resistance provided by the gastrointestinal microbiota. I am the second author of this study. The study was designed by Claudia Eberl and Bärbel Stecher. I conceived and performed the spent media experiments to determine exploitative or interference competition interactions between the OMM^{12} strains, *E. coli* and *S.* Tm. Further, I developed a new protocol to perform phenotypic microarrays for selected strains of the OMM^{12} consortium under anaerobic conditions, as well as the target strains *E. coli* and *S.* Tm. I analysed the data of these experiments and generated the corresponding Figure 4. I contributed to reviewing and editing of the draft manuscript written by Claudia Eberl and Bärbel Stecher.

5.2.2 Manuscript II

The manuscript is available here: https://doi.org/10.1016/j.chom.2021.09.004

5.3 Publication III

5.3.1 Summary and contributions to publication III

In the manuscript "Nutritional and host environments determine community ecology and keystone species in a synthetic gut bacterial community." published on the preprint server bioRxiv and Nature Communications, (Authors: Weiss, A.S., Niedermeier, L.S., Burrichter, A.G., von Strempel, A., Ring, D., Meng, C., Kleigrewe, K, Lincetto, C., Hübner, J., Stecher, B.), we performed a comprehensive analysis of community assembly and ecology of the OMM^{12} synthetic gut bacterial community. I am the sole first author of this study. The study

was designed by me and my supervisor Bärbel Stecher. I developed an adapted protocol of the previously designed community batch culture model, allowing for high throughput screening of community assembly in dropout communities across different cultivation conditions. Together with Lisa Niedermeier, I performed community assembly experiments of all dropout communities across two different cultivation media, generated pH profiles and prepared samples for targeted and untargeted metabolomics analyses. I performed further characterization of communities lacking the identified key species in additional cultivation media, characterization of *Bacteroides caecimuris* I48 polysaccharide degradation in the community context, phenotyping of *Enterococcus faecalis* KB1 enterocin production in the community context and analysis of community level strain relationships. Further, I performed mouse experiments determining community assembly and strain relationships of dropout communities in vivo across different sampling regions of the murine gut and prepared cecal samples for untargeted and targeted metabolomics analyses. I performed the analysis of data sets provided by collaborators Meng and Kleigrewe and generated all figures, except Fig. S5B (identification of polysaccharide utilization loci) and Fig. S7 (spot assays). The draft manuscript was written by me and Lisa Niedermeier. All authors contributed in reviewing and editing the draft manuscript.

5.3.2 Appendix: Manuscript III

The manuscript is available here: https://doi.org/10.1016/j.chom.2021.09.004

In Erinnerung an meine liebe Oma Gretel.

Eine außergewöhnlich erstaunliche Frau voller Lebensmut, die mir in ihrer Stärke, Güte und Entschlossenheit ein großes Vorbild bleiben wird.

Danksagung

Von Herzen möchte ich mich bei **Prof. Dr. Bärbel Stecher** für die großartige Betreuung meiner Doktorarbeit bedanken. Danke für dein freundschaftliches und engagiertes Mentorat, dein stetes Verständnis und deine großzügige Unterstützung, aber ganz besonders auch für dein Vertrauen in mich und meine Arbeit. Die Möglichkeit meinen Forschungsschwerpunkt von der Physik in die Mikrobiologie zu verlegen hat einen ganz grundlegenden Beitrag zu meiner persönlichen und wissenschaftlichen Entwicklung geleistet und ich bin unheimlich dankbar so viel Neues gelernt zu haben und meinen Interessen nachgehen zu dürfen.

A special thanks goes out to my „three friends“ **Alexandra, Benedikt und Marta**. The last years with you were incredibly funny and I am beyond thankful that in you, I not only found smart and helpful colleagues, but also such wonderful friends. With **Lisa** as the perfect addition, I knew every morning coming into our office that whatever good or bad would happen inside and outside of the lab, I would have an amazing group of people by my side.

Nicht unerwähnt darf **Diana Ring** bleiben. Ich werde mir weiterhin immer Urlaub nehmen, wenn du nicht da bist - einfach um sicher zu gehen. Ohne dich geht quasi garnichts im Labor und ich ziehe meinen Hut vor deiner Erfahrung, deinem Fleiß und deinem Replica-Plating Diplom. Danke für deine unermüdliche Unterstützung!
Desweiteren bedanke ich mich ganz herzlich bei allen anderen ehemaligen und aktiven Mitgliedern der Arbeitsgruppe und vorallem bei **Saib Hussain** und dem **Tierhaus-Team** für eure tolle Arbeit und unterstüzung.

Mein ganz besonderer Dank geht an meine lieben Eltern **Joachim und Ulrike**, meine liebe Schwester **Theresa** und mein Spatzl **Jonas**. Danke, dass ihr immer für mich da seid, mich auf meinem Weg unterstützt und euch mit mir über jeden Erfolg freut. Ihr habt entscheidenden Anteil daran, dass ich meinen wissenschaftlichen Interessen nachgehen kann und es bis hierher geschafft habe. Ich bin so dankbar und froh, dass ich euch habe. Ihr seid das Wichtigste und Liebste, was ich hab.