ANNUAL REVIEWS

Annual Review of Animal Biosciences

Synthetic Communities of Gut Microbes for Basic Research and Translational Approaches in Animal Health and Nutrition

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Annu. Rev. Anim. Biosci. 2024. 12:283–300

First published as a Review in Advance on November 14, 2023

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

https://doi.org/10.1146/annurev-animal-021022-025552

Keywords

human microbiome, animal microbiome, health and nutrition, intestinal bacteria, synthetic communities, minimal consortia, live biotherapeutics

Abstract

Microbes and animals have a symbiotic relationship that greatly influences nutrient uptake and animal health. This relationship can be studied using selections of microbes termed synthetic communities, or SynComs. SynComs are used in many different animal hosts, including agricultural animals, to investigate microbial interactions with nutrients and how these affect animal health. The most common host focuses for SynComs are currently mouse and human, from basic mechanistic research through to translational disease models and live biotherapeutic products (LBPs) as treatments. We discuss SynComs used in basic research models and findings that relate to human and animal health and nutrition. Translational use cases of SynComs are discussed, followed by LBPs, especially within the context of agriculture. SynComs still face challenges, such as standardization for reproducibility and contamination risks. However, the future of SynComs is hopeful, especially in the areas of genome-guided SynCom design and custom SynCom-based treatments.

1. INTRODUCTION

The gut is home to billions of microbial cells, with each microbial species covering different roles within their host. For generations we have tried to understand how microscopic organisms influence both health and disease. In 1665, Robert Hooke (1) published *Micrographia*, containing drawings and descriptions of microscopic observations, including "microfungi." A few years later, bacteria, protozoa, and yeast were also observed (2, 3), and we continue to explore microbes and their influence today, including on the animals around us.

Microbes such as bacteria, fungi, and bacteriophages have all been shown to influence animal health (4–6). Even within the intestine of insects such as *Drosophila melanogaster*, bacteria, which are dominant members within host-associated microbiomes, have a symbiotic relationship and are essential for nutrient digestion and host growth (7, 8). Each separate type of bacteria can cover various functions, leading to different effects on their host. Some bacteria are more suited to certain types of nutrients, which can then impact host animal growth (9, 10).

Together, the bacteria and other microbes within an environment such as the gut are called the microbiota (11). The gut microbiota can assist or hinder animal growth (6). The microbiota of each host has evolved over time to more effectively assist animal development (12, 13), with bacteria specialized to its specific host fulfilling ecological niches, better using the host diet. Widespread antibiotic use has also been linked to an increased growth yield in agricultural settings (14) but can also increase methane production and promote antimicrobial resistance (15–17).

The large variance between the microbiota of different animals demonstrates the need to study these microbial ecosystems in a host-specific manner, including the creation of corresponding strain collections. Currently, many bacteria within the gut of both humans and animals are unknown, uncharacterized, or uncultured (18, 19). Study or simulation of such environments can be challenging, with so many unknowns that cannot be accounted for. Similarly, such a large array of variables can greatly influence study outcomes. To reduce the high variance and complexity of gut microbiomes, synthetic communities (SynComs) can be created (see the sidebar titled What Are SynComs?), recreating these complex environments in a more controlled manner, with reduced taxonomic and functional unknowns. Because microbes influence health and nutrition, SynComs can also be created to fulfill therapeutic roles, such as live biotherapeutic products (LBPs) for (*a*) treatment of gastrointestinal infections or noncommunicable disorders such as inflammatory bowel diseases (20, 21) and (*b*) use in the agriculture sector to support a sustainable system with drastically reduced antibiotic consumption by promoting animal growth naturally and preventing

WHAT ARE SYNCOMS?

Synthetic communities (SynComs) are defined as stable mixtures of well-characterized live microorganisms. These normally consist of bacteria but could also include fungi, viruses, and phages (5, 23, 24).

Targeting microorganisms to use within a SynCom can be performed in several ways:

- Bottom-up approach: Collections of cultured microbes are currently the most common method for SynCom creation. Single strains are cultured, selected, then mixed together to form the SynCom. Newer approaches also include the growth of SynComs directly as a mixed culture to enhance yield and stability (22).
- Top-down approach: In this method, a complex community is treated to selectively knock out a substantial proportion of the microorganisms. Constituents of the SynCom are not necessarily individually cultured or taxonomically described and named. However, the community is well-characterized and has been controlled and adjusted to suit a select purpose.

infections. SynComs then provide benefits of fecal microbiota transplant (FMT), without the risk of unknowns or potential harmful or pathogenic components present in fecal samples (22).

In this manuscript we focus on SynComs of gut microbes, starting with SynComs designed for specific hosts. We look at how they can be used in research to address fundamental questions on microbe–microbe and microbe–host interactions, as well as the relationship between microbial communities and nutrition or disease states. For the scope of this article, this has been classified as basic research. Next, we evaluate SynComs as biotherapeutics, especially for agricultural animals, before reviewing current challenges within SynCom research and where we expect SynComs to take us in the near future.

2. HOST-SPECIFIC SYNTHETIC COMMUNITIES

SynComs cover various purposes, dependent on the host species considered. In the 1940s, pigs with vastly different controlled microbiota states were created to study enteric disease. Some pigs were free of any bacteria (termed germ-free or axenic); colonized by known bacteria, defined mixtures, or target complex communities (termed gnotobiotic); or tested for certain pathogens (termed specific pathogen–free) in the hope of removing pathogens that caused diarrhea and mortality. However, it was soon realized that removal of known specified pathogens did not necessarily reduce harm to pigs (25) and that other unknown bacteria within the pigs were causing enteric infections.

Similarly, the ruminant microbiome historically has been explored and controlled via gnotobiotic models to increase understanding of rumination, which is essential to the survival of mature ruminants (26). We now know that, as well as being essential for rumination and meat production (12), the interactions of microbes with different diets can also influence the amount of greenhouse gases emitted by agricultural animals (27).

Attempts have been made to combat animal greenhouse gas emissions using classical probiotics (28). Probiotics traditionally consist of lactic acid bacteria due to their early association with health, rather than direct functionality or condition-specific selection (29). We focus here on SynComs with select constituents targeted for specific functions, purposes, or characteristics, mainly within mice and agricultural animals. Main examples of non-murine-focused SynComs are shown in **Table 1**.

Mice have a long history with SynComs, more commonly tailored to basic research. In the 1960s, a consortium called Schaelder flora was created, consisting of changing sets of anaerobes that were easy to identify under the microscope (45). This consortium would be given to mice that had lived a germ-free life, free of any bacteria prior to colonization. Once colonized, the mice would still be at risk of contamination from non-specified microorganisms and so would be kept in large sterilized containers filled with autoclaved food, bedding, water, food, and filtrated air to isolate them from outside contaminants. It had been noted previously that germ-free animals could not digest many constituents within food, and that SynComs allowed use of added nutrients (46). After further adjustments, the altered Schaedler flora (ASF) was finalized, and it remains a backbone to many SynCom-based experiments. Other reviews (47–49) go into this history in greater depth.

SynComs have many wide-ranging applications, as shown in **Figure 1**. They can be used as tools to discover functions/influences/mechanisms within a host (basic research) and as an aid to disease-specific research and treatment. The next section discusses basic uses of SynComs.

3. BASIC RESEARCH IN MICE

Within basic research, SynComs have been used to aid our understanding of mechanisms/functions within the body, as well as microbe–microbe and microbe–host interactions. SynComs

Table 1 SynComs in nonmurine models[a]

Animal	SynCom information
Drosophila melanogaster	*D. melanogaster* is a classic model organism, first used for breeding experiments in 1906 (30). With a life span of a few weeks, well-characterized physiology, and easy methods for genetic modifications and gnotobiotic derivation in comparison to mammalian models, it is an ideal candidate for basic research on SynComs. *Acetobacter* and *Lactobacillaceae* species typically dominate the *Drosophila* gut microbiome (both wild and laboratory bred). Using this information, a four-member representative consortium has been created (31), which aims to cover essential functions while reducing variance in microbiota-influenced studies. SynCom research on *Drosophila* can also ground further research. Data originally ascertained in *Drosophila* showed that gut microbiota could promote growth in states of malnutrition (32). This enabled further work performed in mice, confirming the importance of microbiota (particularly lactobacilli) in facilitating juvenile growth when malnutritioned (7). Consuegra et al. (9) further built on this work to observe the metabolic cooperation between *Drosophila* and the two dominant gut species, *Acetobacter pomorum* and *Lactiplantibacillus plantarum*.
Bee (*Apis mellifera carnica*)	Bees are key pollinators, making them a vital component of environmental stability and agricultural production. They have also been studied increasingly over the past decade, as climate change and pesticides have been shown to influence bee health and pathogen susceptibility (33). As an animal model, they are also easier to work with compared to mammalian counterparts, both in terms of time (life span) and within the ethical bounds of the 3Rs (replacement, reduction, refinement). SynComs have allowed researchers to dissect the role of the bee microbiota in their disease susceptibility, ability to use nutrients, and cognition. A bee SynCom consisting of seven bacterial strains (34) has given insight into the metabolism of pollen via bacteria and the effect of both bacteria and metabolites on the host. More recently, the effect of bacteria on memory and cognition within bees has also been highlighted (35). The information gained from the use of bee SynComs may allow us to better understand and manipulate host–microbe interactions, allowing bees to survive and thrive in the future.
Mosquitos	Mosquitos affect large sections of the world and environment, including transmitting diseases such as malaria. The colonization of anopheline mosquitoes with a bacterial symbiont of the genus *Delftia* was recently shown to suppress malaria transmission, highlighting the importance of studying microbe–host interactions in this host species (36). Whether mosquitos can survive the larval stage without microbiota is subject to debate. One approach to avoid this is transient colonization with *Escherichia coli* that can be knocked out, facilitating the creation of germ-free mosquitos (37). To improve this model, research on mosquitos and their life cycle is now being facilitated using SynComs. A three-member community of bacteria (38) was compared to germ-free, heat-treated *E. coli* and conventionally colonized mosquitos for survival to adulthood and growth. Growth was comparable to conventionally colonized mosquitos, as was survival to adulthood. Inactivated *E. coli*–treated mosquitos suffered from stunted growth but lived longer on average compared to untreated mosquitos.
Caenorhabditis elegans	*C. elegans* is another well-studied model organism, due to their transparent anatomy, simplified physiology, short life cycle, and available genetic tools (39). While *C. elegans* are raised by grazing on laboratory strains of *E. coli*, the impact of alternative grazing bacterial species on *C. elegans* growth has been evaluated only recently (10). Samuel et al. (10) collected varying *C. elegans* food sources (oranges, apples, cactus fruit, snails, black bryony), analyzed the microbiota in each environment, and created SynComs (18–24 strains) based on the bacteria found. This led to the observation that 80% of bacteria found in the food sources were assistive to growth. More recently, CeMbio, a *C. elegans*–based SynCom, has been created (40) using characterized and genome-annotated bacterial strains originating from *C. elegans* to create a representative community.

(*Continued*)

Table 1 (*Continued*)

Animal	SynCom information
Zebrafish	Zebrafish are a common invertebrate model, used commonly in toxicology/therapeutic compound screenings but also for disease modeling and basic research (41). A 37-strain SynCom has been created to protect against pathogens, using common bacterial strains used in the food industry (42). Different pathogenic bacteria were screened, with *Edwardsiella ictaluri* chosen as the pathogen with the highest mortality rate in mono-colonized zebrafish. The 37-strain SynCom was successful in increasing survival rates when larvae were challenged using *E. ictaluri*. Of the 37 strains, *E. coli* was found to be particularly protective. Looking further into the mechanism revealed that adhesion factors such as F-pili influenced the SynCom colonization.
Pig	The pig is often used as an animal model within science due to its similarity to humans in physiology, size, and overall anatomy. Adult porcine models of the human microbiome also engraft better than their mouse counterparts (43). Pigs are also vital within the food/agricultural industry, making them good in vivo models for SynComs. To create porcine SynComs, bacterial isolates from pig can be used, such as those within the pig intestinal bacterial collection (18). So far, SynComs within pigs have been limited, although several have been produced. In 2011, it was discovered that a prenatal SynCom of nine bacterial species (also fed to preweaning piglets) created a long-lasting change in gut microbiota and short-chain fatty acid composition within piglets, even after 1 month of normal diet. Another SynCom, Bristol microbiota (44), has been created to reduce mortality of neonatal piglets by stimulating immunoglobulin production in early life using bacterial consortia that reliably colonize. More can be read on pig gut microbial targets at Reference 17.

[a]In addition to the important mouse-/human-based SynComs discussed in depth in this review, SynComs are also created in different model animals.
Abbreviation: SynCom, synthetic community.

often have been used in conjunction with rodent models such as the rat and mouse, two of the most common experimental models of mammals.

3.1. Altered Schaedler Flora

As briefly stated above, one of the first SynComs to contribute to basic research was the altered Schaedler flora (ASF). ASF has been a cornerstone to the scientific community, spanning six decades of gnotobiotic research. Initial studies using an earlier iteration, Schaedler flora, focused on how nutrition/diet composition could influence growth of certain bacteria, as well as increase their resistance to colonization from pathogenic *Staphylococcus* and *Klebsiella* species (45, 50, 51).

Later studies also showed ASF to have a protective effect compared to germ-free mice, reducing mortality in mice infected with *Clostridium botulinum* (52). ASF was also used to confirm that DNT (dinitrotoluene, a carcinogen used within liver research) required metabolism by intestinal microbes to produce carcinogenic effects (53).

ASF has allowed us to better understand the influence of gut microbiota on the immune system and early-life immune development. Transient colonization of pregnant mice (either germ free or ASF colonized) with a genetically engineered *Escherichia coli* increased group 3 innate lymphoid cells (ILC3s) in pups (known to influence inflammation and intestinal barrier function) but did not affect the B or T cell population compared to non-transiently colonized controls (54). This was followed by experiments swapping pups at birth to detect whether maternal antibodies (immunoglobulin G) transferred through milk influenced the ILC3 increase. It was found that IgG had to be transferred both across the placenta and through milk to produce significant effects. This application of ASF shows an alternative use, whereby SynComs can be used as background or control communities emulating a complex microbiota without variance of unknowns. (Additional uses of ASF are also discussed in **Table 2**.)

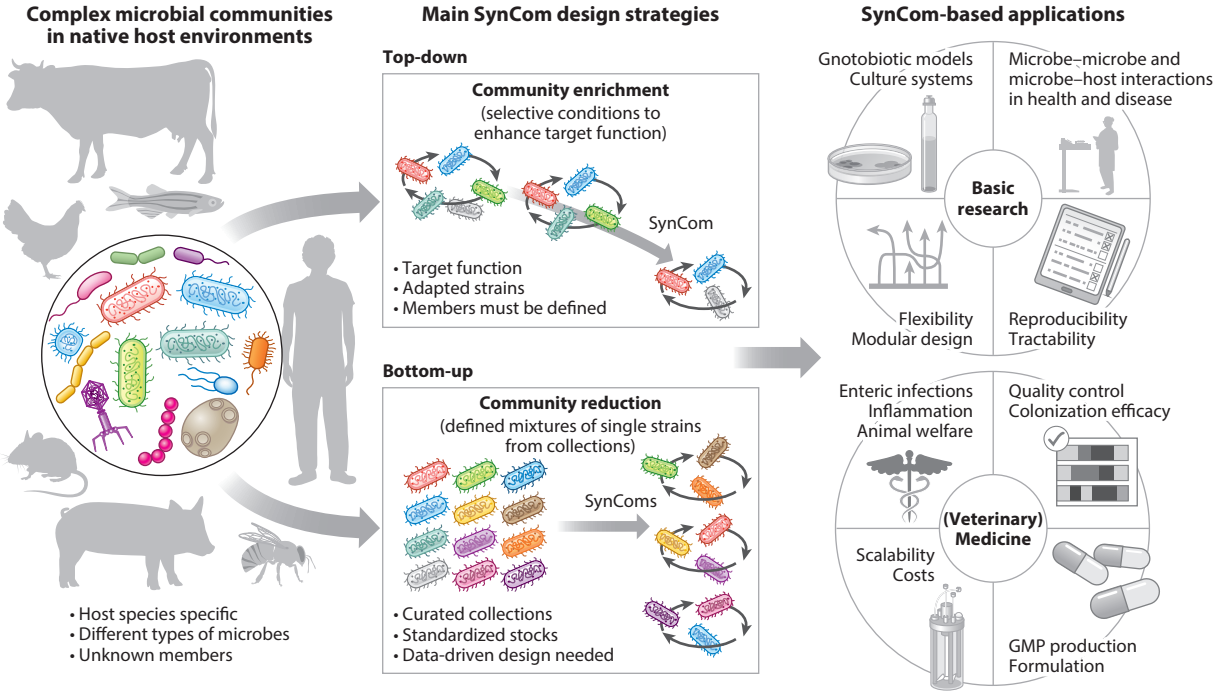

Figure 1

Possible methods for SynCom creation, use, and considerations. SynComs can be used for both basic research and therapeutics, for animals and humans. They can be made through either a top-down approach (enrichment of specific microbes from a complex environment) or a bottom-up approach (specific strains picked and mixed together for purpose). Use case will influence strain suitability, including host specificity. Basic research includes SynComs that enhance physiological and mechanistic knowledge, metabolic understanding, microbial influence on host organs, and disease states. They can be used in culture systems or gnotobiotic animals. More therapeutic uses of SynComs include protection against diseases such as infections and maximizing nutritional uptake and growth. SynComs used for basic research or therapeutic purposes must fulfill different criteria, as highlighted within the circles (*right panel*). Abbreviations: GMP, good manufacturing practice; SynCom, synthetic community.

3.2. Oligo-Mouse Microbiota

The Oligo-Mouse Microbiota (OMM) model was created in the last decade with the original aim to reduce *Salmonella* infection. The original OMM[12] consortium (which included 12 phylogenetically diverse bacterial strains from the mouse intestine) was supplemented with additional facultative anaerobes to maximize *Salmonella* colonization resistance (55). OMM covers a wide variety of phyla and functions and has been used to delve deeper into microbe–microbe interactions and metabolic networks between the community members (16).

More recently, an extension OMM[19.1] with additional bacteria (19 strains in total) and functions has been created (56). This has further increased the consortium's functionality while still controlling the environment. OMM[19.1] ameliorates germ-free phenotypes such as reduced lung and heart volume to conventional levels. Regulatory T cells and T helper 17 cells within the gut of OMM[19.1] mice have been increased to equalize levels seen in conventional mice and have also been shown to recover bone density—normally reduced in germ-free mice—to levels seen in conventional mice.

Because the OMM SynComs are publicly available, they can be adjusted. Van Tilburg Bernardes et al. (23), for example, used OMM[12] plus fungi to study fungi interactions between microbiome and host. They showed that fungal–bacterial interaction exacerbated colonic

inflammation in dextran sulfate sodium–induced colitis and also promoted immune activity in early life. Additional uses of Oligo-MM are also discussed in **Table 2**.

3.3. GM15

GM15, a mixture of 15 bacteria from the mouse gut, including isolates from the mouse intestinal bacterial collection (19), also looked at immune parameters, focusing on serum readouts (57). Similarly to OMM, it aimed to cover a wide variety of functions, including enzymatic activities such as riboflavin, short-chain fatty acid (SCFA), and glutathione metabolism. Mouse physiology over generations was analyzed, and growth markers such as femur length were recovered to conventional levels in GM15 mice. Interestingly, when both GM15 and OMM^{12} mice were subjected to postweaning malnutrition, those colonized with GM15 or OMM^{12} maintained an increased body weight, size, and femur length compared to conventional mice; specific phenotypic differences (e.g., fat tissue, bone growth) were nonetheless observed between the two SynComs.

3.4. Complex Murine SynCom

All the above examples used communities of rather low complexity to emulate the functions of a native ecosystem. Although this substantially simplifies the logistical aspects of experiments, it recapitulates only a small fraction of the original diversity and landscape of microbial functions. In 1970, a consortium of 120 mouse strains was published with the aim of recapitulating normal phenotypes in mice (58). Mice were colonized with 50 strict anaerobes and 80 facultative anaerobes, and normality was confirmed using parameters such as cecum size, intestinal histology, and level of *E. coli* engraftment after colonization.

3.5. Human-Based Mouse Models

So far, the SynComs discussed have been designed for a specific host, containing specific strains isolated from the same host. However, in the early 2010s, a human strain–based SynCom, SIHUMI, was created and placed into rats, with a focus on the human gut microbiome (59). SIHUMI successfully colonized and formed SCFAs more similar to human levels compared to conventional rats. SIHUMI has also been used to study the immune system. Eun et al. (60) showed that SIHUMI can induce antigen-specific colitis in a mouse model.

Human-based SynComs allow for translational observations of interactions and products of the human microbiome. However, bacteria placed into non-native hosts may not be suited to the ecosystem it is place into, or fulfill the niches present (59, 61, 62). This was observed with SIHUMI, where relative abundances of the bacteria throughout the gut from ileum to colon did not change, suggesting a lack of functional specialization to the different gut areas (60). A study of different *Limosilactobacillus reuteri* strains further supported this principle, showing that *L. reuteri* strains vary between different animal hosts and that host-specific *L. reuteri* strains contain genes suited to host-specific mechanisms (63).

There have also been attempts to create larger, more complex synthetic human communities; hCom2 (119 bacterial strains community) (64) was built to fulfill the empty functional niches of the original hCom using a fecal challenge. Mice colonized with hCom2 have physiological phenotypes similar to those colonized with human feces. (hCom2 is discussed in greater depth in Section 5.)

3.6. Targeted SynCom

Over the last decade, creation of SynComs has become more attainable with advances in techniques and a greater understanding of bacterial processes, due to improvements in sequencing

(65). SynComs can be designed in a targeted manner to better represent specific phenotypes, such as disease environments or unknown mechanisms. Examples include communities made of either 9, 14, or 35 bacteria, created to gain insight into trypsin degradation, a suggested route to regulate inflammatory bowel diseases (66, 67). Bacteria may reduce trypsin levels, leading to increased IgA levels and improving the effectiveness of oral vaccines against pathogens (66).

Another major group of metabolites produced by gut microbiota are SCFAs. Although not tested in mice, SynComs have been designed specifically to maximize production of the SCFA butyrate (68).

The interactions between different types of microbes within the gut have also been observed using SynComs. Using a 15-member community, Reyes et al. (24) studied phage–bacteria dynamics. They confirmed that some bacterial host species were resistant to phage attack and that changes in the abundance within the community due to attack tended to be temporary, recovering over time.

SynComs can also be created to replicate stages of development, such as the microbiomes of children and preweaned mice in early life. PedsCom simulates a young immune system, enabling study of diseases more susceptible for a preweaning microbiome, such as *Salmonella*, even once mice have been weaned and progressed to adulthood (69). Such work shows the critical nature of early-life microbiome exposure for immune maturation.

3.7. SynCom Influence on Disease

SynComs (those described above but also others) have been used to specifically study microbial influences on disease states, as shown in **Table 2**.

4. SYNCOMS AS LIVE BIOTHERAPEUTIC PRODUCTS

Besides the use of SynComs in translational animal models to study molecular mechanisms underlying diseases, as detailed in the previous section, SynComs have already been used in clinical settings and in the agricultural sector. These are referred to as live biotherapeutic products (LBPs), which can be defined as preparations that contain live microorganisms to prevent or treat diseases. For clinical use, LBPs fall under drug product legislation, which adds multiple layers of complexity to their design and use (78).

Study of the role of live microorganisms in sustaining health dates back from the early 1900s and the concept that fermented milk products benefit the host (79). The original concept of probiotics, mostly restricted to a limited diversity of yeasts or lactic acid bacteria, was coined much later, in the late 1980s, primarily for use in animals (80). Since then, the concept has evolved substantially (81), driven by the need to specifically use microbes selected for a target function and not merely use a limited number of strains for multiple applications due to their general status as safe bacteria for human or animal consumption. This transition has been facilitated by the renewed interest in anaerobic cultivation, which enhances the throughput of bacterial isolation and the identification of strains with probiotic potential. Hence, the breadth of bacterial diversity that can be considered for use as LBPs has been extended to commensal bacteria colonizing the intestine of healthy donors. The most prominent example of such a bacterium is *Akkermansia muciniphila*, which was shown to have beneficial effects on metabolic health (82). Of note, 15–20 years were required from the first description of this bacterium in 2002 until interventional use in human subjects and the identification of a bioactive molecule from its cell wall (83–85). To date, the use of single microbes has been the preferred strategy due to ease of handling/manufacturing and to having a direct link between a defined species and pathophysiological targets. However, an emerging paradigm based on ecological principles within microbial communities is that mixtures of strains that synergize

Table 2 SynCom use cases related to host health

Use case	SynCom
Behavior	Use of SynComs has facilitated research into the influence of the gut microbiota on behavior. Lyte et al. (70) showed that ASF promotes anxiogenic behavior compared to SPF mice in Open Field and Elevated Plus Maze experiments. Precursors of serotonin, histamine, and dopamine were altered between ASF and SPF groups; this has been suggested as a possible mechanism.
IBD	Microbiota have been shown to influence IBD. *Enterococcus faecalis* (a commensal species within both humans and rodents) can protect against worsening colitis in the presence of SIHUMI (71). This is in contrast to experiments using mono-colonization with *E. faecalis* that showed an exacerbation of colitis symptoms, highlighting the importance of SynComs (71).
	The influence of viruses on IBD has also been studied, using OMM^{12} or ASF as a SynCom backbone (72). MNV can induce colitis in IL10-deficient mice. OMM^{12}-colonized mice did not have exacerbated colitis when infected with MNV. In contrast, MNV exacerbated colitis symptoms in ASF-colonized mice. When ASF-colonized mice were co-colonized with SFB, colitis symptoms were not exacerbated, suggesting a protective effect of SFB (as well as other bacteria present in OMM^{12}) to MNV severity.
	A rationally designed human consortium is GUT108, consisting of 11 bacteria. It has been proposed as a treatment for immune-mediated colitis using multiple butyrate-producing clostridia, shown to decrease colitis severity (20).
Immune response	Immune responses to microbiota can be studied in greater depth using SynCom. ASF has been shown to induce more regulatory T cells in mice compared to germ-free (73), showing that the microbiota can influence proportions of T cells.
	ASF also can play a protective role in mesenteric ischemia-reperfusion injury by increasing leukocyte adhesion (74).
	OMM^{12} has been used to study B-cell dynamics (75). Germinal centers (within the gut) are normally hubs for targeted antibody responses from B cells for infections. When comparing germ-free and SPF-colonized mice, B-cell clonotype dynamics differ in the presence or absence of bacteria. Colonization of germ-free mice with OMM^{12} showed that bacterial colonization could recover/induce some, but not all, germ-free dynamics.
Obesity	Obesity is a disease that is becoming an increasing issue within society. Microbiota plays a role in high-fat diet–induced obesity (76). High-fat diets were fed to mice colonized with SIHUMI in the absence or presence of *Thomasclavelia ramosa* (formerly *Clostridium ramosum*). *T. ramosa*–colonized mice had increased body weight and fat, linked to upregulated fat-uptake transporters in the small intestine.
Protection against pathogens	A murine rationally designed consortium (77) has been used to show how microbiota can protect hosts from opportunistic pathogens (i.e., *Clostridioides difficile*). The consortium was designed to use mucus-derived sugars, to compete against pathogens that use mucosal sugars as a vital nutrient source.

Abbreviations: ASF, altered Schaedler flora; IBD, inflammatory bowel disease; MNV, murine norovirus; SFB, segmented filamentous bacteria; SPF, specified pathogen free; SynCom, synthetic community.

can be used to enhance the desired effect and even increase stability, and thus colonization efficacy, of the strains within the products. Moreover, due to general loss of microbial diversity in multiple diseased conditions and the therapeutic success of FMT, especially in the context of enteric infections, such as by *Clostridioides difficile*, mixtures of strains are considered an enhanced strategy to recapitulate FMT products under controlled conditions. The aim is to increase safety, reproducibility, and efficacy, albeit with multiple additional challenges (see next section), partly due to the legislation for drug products that applies to LBPs, as mentioned above. Currently, LBP-based applications in the microbiome field are blooming, and research centers dedicated to their

investigation have been established (86). This is paralleled by an even greater interest in the industrial sector, with multiple start-up and higher-scale companies working actively on this topic.

Until recently, there had been three reports of LBPs containing a low to moderate diversity of bacteria (8 to 33 strains) used to successfully treat *C. difficile* infection in a limited number of human patients (2–8, 21, 87, 88). The LBP used in the study by Dsouza et al. (21), namely VE303, which contains eight bacterial strains, was recently reported to show efficacy at a dose of 8×10^9 CFUs daily in a phase 2 trial with 79 participants (89). Multiple additional SynComs for clinical use are being developed based on preclinical studies, such as those studying the stimulation of T regulatory cells by cocktails of butyrate-producing clostridia (90, 91). However, issues related to patent law, which is often implemented for LBPs due to their commercial applications, delay public access to findings in clinical settings.

Similar to the work in humans detailed above, LBPs are being developed and tested for use in farming to improve animal health, e.g., to enhance resistance to enteric colonization by pathogens such as *Enterobacteriaceae*, *Clostridium perfringens*, or *Salmonella* (as for *C. difficile* in human) and to support feed digestibility or animal growth. The aim is to reduce the use of antimicrobial substances, improve animal well-being, and generate positive environmental effects (e.g., reduced methane production). Having less, albeit different constraints associated with the production of LBPs for animal use may facilitate their implementation. Very few LBPs for animal use have yet been released, in part due to the lack of specific isolates available for each of the target animal species. Species-specific isolates are better suited to their original host (as stated in the previous sections), due to diet and physiology. Hence, establishing curated collections of well-characterized, animal species–specific isolates is essential to facilitate LBP creation. Foundations for such collections have been established for the cow rumen (92) and the intestine of chicken (93–96) and pigs (18).

Recent examples of research studies for the use of LBPs in animals include a mixture of nine phylogenetically and functionally diverse bacterial strains designed to stimulate the adaptive immune system when provided to chickens early in life (94). One asset of this study is the public availability of the strains, which enables the results to be reproduced and the work to be continued by others (**https://www.dsmz.de/chibac**). Another study showed that single bacterial strains isolated from the chicken gut antagonized colonization by *Salmonella enterica* serovar Enteritidis only moderately, emphasizing the need to study the effects of cocktails including multiple strains (97). In pigs, despite a public repository of strains from their intestine (18), the acknowledged effects of their gut microbiome on body growth (98), and the important health and environmental issues directly linked to postweaning diarrhea and associated alterations in the gut microbiota (17), no comprehensive study has yet been published on the design and efficacy of LBPs for use in this common domestic species. In ruminants, LBPs can be used to stimulate dietary fiber breakdown within the gut and modulate microbe–microbe and microbe–host interactions that facilitate nutritional digestion. This has been of interest for decades (99), but again no LBPs for use in ruminants have been developed and studied extensively to date. Recent studies have shown differences between the gut microbiome of cows characterized by high feed efficiency/low methane emission versus low feed efficiency/high methane emission (27). Other studies based on enrichment methodologies to generate and investigate SynComs have highlighted the importance of fungi–archaea interactions for methane production in the cow and goat intestine (100, 101). Given renewed interest in the cultivation of microbes from the cow intestine (92), LBPs that can be used to modulate the rumen ecosystem toward beneficial functions to both improve animal health and reduce burden on the environment will most likely be developed in the very near future.

In summary, research and applications based on LBPs using commensal microbes isolated from the intestine of the corresponding host species is a very promising field. However, it is

still in its infancy, especially in livestock. More efforts are needed to cultivate, characterize, and archive commensal microbes and to develop innovative strategies to design and produce stable LBPs with the desired function. Currently funded research and training programs, including MonoGutHealth (**https://monoguthealth.eu**), HealthyLivestock (**https://healthylivestock.net**), the PIG-PARADIGM project (Novo Nordisk foundation), and RuMinimum (**https://cordis.europa.eu/project/id/866530**), will certainly deliver valuable insights toward these goals soon.

5. CHALLENGES AND THE FUTURE OF SYNCOMS

Research and applications using SynComs are flourishing, but important aspects must be considered to continue making meaningful progress. Genetic drift can occur within a given strain over time, with decade-old ASF breeding isolators very likely having strains different to those originally used in the 1960s. This is supported not only by comprehensive in vitro data on strain evolution in *E. coli* (102) but also by recent mouse experiments using the reference mouse SynCom OMM^{12} (103). The latter study reported mutation rates and associated positive selection processes over a period of six years, showing long-term evolution and coexistence of substrains within an individual ecosystem; these substrains responded differently to changes in the intestinal environment, e.g., due to diet. This clearly shows that standardized stocks, as recently proposed for OligoMM models (56), are an important resource to maintain the quality of SynComs in the long term, both to ensure the reproducibility of research models and to maintain the desired function and thus efficacy of LBPs for treatment purposes.

Differences in colonization strategies (e.g., live cultures or frozen mixtures, medium formulation, co-housing, water supplementation, gastro/rectal gavage) and varying conditions between animal facilities (e.g., drinking water, feed, hygiene measures) also can lead to different stable colonization outcomes. The method used to test colonization (e.g., gene-targeted or shotgun sequencing, strain-specific quantitative polymerase chain reaction, cultivation) also influences our snapshot views of the real communities. Nevertheless, studies have demonstrated reproducible colonization of reference SynComs in multiple facilities and across several generations of mice, at least for dominant members of the communities (56, 104). Whereas lowly abundant strains are more prone to artefacts from the detection methods, some of the dominant members of SynComs may also be missing, depending on the colonization strategy (see above) and the equipment available to users for working with strict anaerobes that are not aerotolerant. For those bacteria, strain intake frequency can be important. Multiple applications or repeated feeding was shown to enhance the chance to obtain reproducible colonization profiles (104). This may prime the gut environment with added nutrients and environmental changes (such as pH, partial pressure of oxygen) to become a suitable environment for more fastidious bacteria to colonize. The frequency of strain intake is likely to be even more important in the case of LBPs used to treat patients or animals that are already colonized by endogenous communities of microbes, even if these communities are disturbed. Colonization at different time periods in a host animal's life, such as pre- or postweaning, can also change colonization efficacy and effects on the host (105), which is again relevant in the case of both research models and LBPs.

Most SynComs for research or treatment purposes are of low complexity (<10–15 strains). Low complexity enables the amendment of basic SynComs with specific strains of desired functions as multiple niches within the community remain to be filled. Hence, working with reference communities of low complexity both is easier technically and provides a backbone for flexible experiments adapted to the specific needs of a given study. However, although better than monocolonization with a single specific strain, low-complexity SynComs are still gross approximations

of native ecosystems and prone to contamination (engraftment of undesired strains). Moreover, low-complexity LBPs might not be as efficacious as products of higher complexity. Recently, a SynCom of 119 strains, hCom2, was developed (64). Specifically, a first version of it, hCom, was improved by identifying intruders from a fecal challenge and recapitulating these microbes into the improved hCom2. Working with such a large community of bacteria is technically challenging due to the difficulty of recording colonization of the individual strains, which complicates reproducibility. Moreover, the microbes originated from multiple donors, leaving room for improvement toward individualized SynComs (see below). In general, it is very important that strains included in SynComs are very well characterized (and named, in the case of novel taxa). Their tractability is essential for meaningful long-term use; this is hampered by the fact that strains are very often not publicly available or cannot be accessed due to lack of deposition at international collections. The naming of SynComs themselves can also generate confusion and could benefit from simple guidelines. For instance, multiple versions (i.e., strain combinations) of the original human SynCom SIHUMI created by Blaut and colleagues (59) were published (76, 106), and Stecher and colleagues' original OMM^{12} model (55) has also been published under the name sDMDMm2 (107, 108).

Safety authorities have begun to approve the transfer of whole fecal microbial communities for human use, and it will soon be the microbiome-based method of choice for the treatment of otherwise incurable diseases, such as gastrointestinal infections and inflammation. In animals, although several species practice FMT naturally via coprophagy at different periods of their lives, drastic hygiene measures in farming antagonize this natural process, and defined products such as probiotics are preferred for veterinary use. In both cases (humans and animals), microbiome-based applications must be developed further. In research, a few reference SynComs exist, and some of them (ASF, OMM) have been used more broadly. However, the diversity of research projects is broad, and reference SynComs are not always adapted, leading to the creation of unrelated, study-specific models. Hence, current work with SynComs represents only a small fraction of what is possible. We predict that 10 years from now, it will be possible to easily create and use standardized SynComs that are customized for individual purposes. For this to happen, however, progress must continue on multiple fronts.

First, more isolates are needed, and they must be well characterized (genomically and phenotypically), validly named in the case of novel taxa, and archived under state-of-the-art conditions at multiple international collections. As emphasized before, strain catalogs must be established in a host species–specific manner to guarantee that the strains being used are taxonomically and functionally meaningful. This requires a substantial increase in funding for cultivation activities that have been neglected in the past 20 years of sequencing-based research. Broad access to strains is essential for research purposes. For commercial use, proprietary issues antagonize such goals in the first place. However, detailed information on the strains is mandatory for patents, as well as its intended use, e.g., *Faecalibacterium prausnitzii* and *Desulfovibrio piger* for use in treating or preventing diabetes and bowel diseases (109). Safe deposit options exist in international collections, and the depositors retain the rights to decide on further use of the strains for commercial purposes. Hence, high-quality archiving of microbial strains outside the walls of single universities or companies must become a priority.

Second, SynCom design has so far mainly followed community reduction approaches based on expert knowledge of the taxonomy and broad functionality of species that can be cultured (110). Data-driven, computer-guided SynCom design is needed to streamline the selection of strains based on their functional capacities, e.g., cover a maximum of functionalities from the original complex ecosystem to be matched or complement missing functionalities within a disturbed ecosystem. Examples exist, and efforts in this direction must be continued (67, 111–113).

Such endeavors must include the use of metabolic models to better appreciate the landscape of interactions within communities and possibly infer their stability, enabling choice of an optimal SynCom among different possible compositions that may provide the same functional benefit. Experimental data focused on lignocellulose-degrading communities from the horse gut suggest that strains grown within SynComs selected by community enrichment from one original native community perform better than strains of target species cultured separately and put together (100). This suggests that metabolic synergies between strains that coexist in natural environments (and by extension in enriched communities) are important, which opens avenues for two future strategies. The first is the creation of personalized collections of microbes isolated from one single sample to reconstruct low- to high-complexity communities using strains that are used to coexisting. Advanced technologies now enable the high cultivation throughout that is required to do this (114). The second is high-throughput generation of random communities from any given sample (e.g., by single-cell sorting), followed by function-based selection of most effective SynComs. Such an approach most likely is limited to metabolic function–based selection, for which rapid assays can be implemented to select the best SynComs. This requires detailed downstream functional characterization of the communities in mind, as well as characterization of single members to obtain a defined product. This highlights the importance of target functions of a SynCom, which can be more paramount than its bacterial composition.

Third, regarding SynCom use in the clinic or on farms, manufacturing processes and their costs play a very important role. Producing multiplexed cultures of commensal species, including fastidious anaerobes, under good manufacturing practice (GMP) conditions is a challenge, not to mention the difficulty of defining the final formulation of products and the need to develop scalable workflows. Production strategies based on the direct cultivation of microbial consortia seem promising, as proposed recently (22). However, detailed quantification of single strains within a LBP grown directly as a consortium to guarantee product quality as per regulatory standards becomes problematic with increasing diversity. Moreover, such regulatory hurdles vary depending on the country.

Fourth, whereas major efforts focus currently on bacterial members of gut microbial communities, we expect the inclusion of viruses, archaea, and fungi in SynComs in the near future. This has been tried already in a research context to study interactions between phages and their bacterial hosts (5, 24) and is particularly relevant in certain ecosystems, such as the herbivore gut microbiome (e.g., the role of fungi and archaea in methane production). With these major elements for future developments in mind, SynComs of gut microbes have a bright future for both research and applications in clinical and agricultural settings.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

T.C. is funded by (*a*) the German Research Foundation, project ID 395357507–SFB1371 "Microbiome signatures" and project ID 403224013–SFB1382 "Gut-liver axis" and (*b*) the Federal Ministry of Food and Agriculture, project FKZ 281C402C21–ChiSyn.

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