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Minireview

Engineering the microbiome for animal health and conservation

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Impact statement

Considering the clear effects of microbiota on important aspects of animal biology and development (including in humans), this topic is timely and broadly appealing, as it compels us to consider the possibilities of altering the microbiome (without antibiotics) to positively affect animal health. In this review, we highlight three general approaches to manipulating the microbiome that have demonstrated success and promise for use in animal health. We also point out knowledge gaps where further inquiry would most benefit the field. Our paper not only provides a short and digestible overview of the current state of application, but also calls for further exploration of the microbial diversity at hand to expand our toolkit, while also leveraging the diversity and flexibility of animal systems to better understand mechanisms of efficacy.

Abstract

Interest in animal microbiomes as therapeutics is rapidly expanding, as techniques to study the microbial world decrease in cost and increase in accessibility and case studies from human medicine receive widespread attention. In this review, we summarize the current state of techniques to modify the microbiome to improve animal health, focusing on applications in domestic pets, farm animals, and in wild settings for conservation. We discuss options for modifying the microbiome, including community-wide changes such as fecal microbiota transplants, prebiotics, probiotics, and antibiotics, and more targeted approaches such as phage therapy and CRISPR-Cas. We conclude that although much remains to be done in untangling the basic biology of microbiome-directed therapies in animals, the rapid progress currently being made in human medicine and the examples to date of application of probiotics and other microbiome-directed therapies in taxa ranging from horses to salamanders to bees suggest excellent prospects for these technologies as they are further developed and as data on both the benefits and risks are carefully and systematically collected.

Keywords: Probiotic, microbiota transplant, microbiome, animal health, disease, dysbiosis

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Introduction

All animals are home to microbes (bacteria, archaea, fungi, microbial eukaryotes, and arguably viruses), the collection of which is now often referred to as the microbiome. Faster and cheaper DNA sequencing and data analysis pipelines over the past two decades have enabled a surge of investigations into the microbiome and its role in health and disease, including host nutrition, metabolism, development, immune function, and behavior. One primary goal of these studies is to use the microbiome as a biomarker for disease and health conditions, as well as a way to treat these conditions (i.e. personalized medicine). Research into these topics with a focus on human health has paved the way for applications suitable for animal health. However, the

potential for expedited discovery of effective applications in animal systems is greater than in humans in several ways, because diet and host genetic diversity are often more standardized (at least in domesticated and captive animals), and animal systems can often be more easily manipulated experimentally. Finally, privacy regulations (e.g. HIPAA) are typically less burdensome than for human research, so data capture and sharing, especially of detailed and identifying medical records, images and sensor data, are far more feasible.

Targeting microbiome health in livestock, pets, and wildlife will each have significant economic and societal impacts. With a growing world population reliant on animals and their products (estimated to be 10 billion people by the year 2050 according to a 2017 United Nations report[1]), combined with increasing pressure to reduce antibiotic use in foods, incentives to leverage the microbiome as a tool to promote healthier and more productive livestock are now greater than ever. Indeed, a growing body of research indicates that production can be increased while making it less reliant on antibiotics, and the microbiome has also shown promise as an effective target in the areas of pet health and animal conservation, helping to reduce morbidity and combat infectious diseases.

The first step towards developing effective treatments is identification of a health issue to treat. However, this can be a nebulous task, and questions regarding how well we can assess animal health by reading out the microbiome are topics of much debate. Although infection by individual pathogens is often apparent and easily tested, for example by PCR or mass spectrometry-based assays, assessment of overall microbiome health is more difficult. However, research shows that microbial dysbiosis, described as an "imbalance" of the typical microbiota caused by perturbation, is often linked to negative health conditions. Dysbiosis could arise a number of ways: an overgrowth of pathogenic microbes (or opportunistic pathogens), a depletion or absence of beneficial microbes, or an overall decrease in microbial diversity[2]. However, definitions of which microbiome perturbations represent dysbiosis can vary from system to system and from individual to individual[3] (see Box 1). In some systems, antibiotics clearly have far-reaching effects beyond their impact on the pathogen they are administered to treat. Antibiotics can eliminate beneficial microbes along with target pathogens, cause dysbiosis and lead to negative health outcomes. For example, in bees, treatment with antibiotics greatly reduces the total bacterial abundance and absolute abundance of several core bacterial taxa, and leads to increased mortality.[12] Similar evidence for antimicrobials disrupting the gut microbiota has been reported in horses,[13,14] chickens,[15] dogs,[16] and cats,[17,18] although treatment does not always result in negative clinical symptoms. In other situations, the mechanism by which dysbiosis occurs may be less obvious; nonetheless the connection between the microbiome and health is evident. For example, captive primates prone to gastrointestinal problems have been shown to have a gut microbiome different from healthier primates,[19] and dogs with inflammatory bowel disease (IBD) show indications of a dysbiotic microbiome, although in ways that are different from humans with IBD.[20] In horses with colitis, differences in the abundances of certain microbiota rather than differences in their presence or absence suggest that colitis may also be a disease related to gut dysbiosis.[21]

Box 1 Infection-induced dysbiosis or adaptive shifts in the microbiome?

In addition to perturbations of the microbiome such as antibiotics or environmental stress from pollutants or habitat alterations, disease is included in the definition of dysbiosis as both a cause and a consequence.[2] This can be problematic because a shift in the microbiome in response to infection may in some cases represent an adaptation. Adaptive shifts in the microbiome may represent a form of immune response in which microbes that can compete with invaders are favored. Dysbiosis may thus be more appropriately defined as a decrease in microbiome stability.[3] For example, microbial diversity often decreases in amphibians upon infection with *Batrachochytrium dendrobatidis.*[4–6] As competitive members come to dominate, the microbiome does not necessarily become dysbiotic, just as the host does not always become morbid. Whether the hosts survive or succumb to disease may be in part a result of the rate of microbiome response to pathogen proliferation (Figure 1(a)). Similarly, beneficial shifts in the microbiome may result from manipulation of the microbiome (Figure 1(b)), and may be preventative or restorative depending on the goal of treatment.

Studies have shown that manipulation and bioengineering of the microbiome can be a powerful tool in reshaping and stabilizing (and in some cases, restoring) a dysbiotic community. Here, we highlight three general approaches—culture-based supplementation, community-level supplementation, and selective targeting—that have been shown effective in improving animal health (Figure 2), as well as potential future therapeutics. There is already extensive literature on the use of antibiotics as a modulator of the microbiome, and decades of widespread use of antibiotics in livestock and aquaculture is raising safety concerns due to links to increasing antibiotic resistance. This problem is compounded by the fact that anaerobic gut commensals in humans have been shown to have the *vanB* locus encoding for vancomycin resistance,[22] highlighting unexpected outcomes of using antibiotics to treat livestock on a massive scale. Therefore, antibiotics will not be a focus in this review, but rather alternative approaches to engineering the microbiome.

Supplementation to enhance growth/activity of specific beneficial microbes

One of the fastest growing areas of animal microbiome research is in developing probiotics (defined by ISAPP as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[23,24]) and

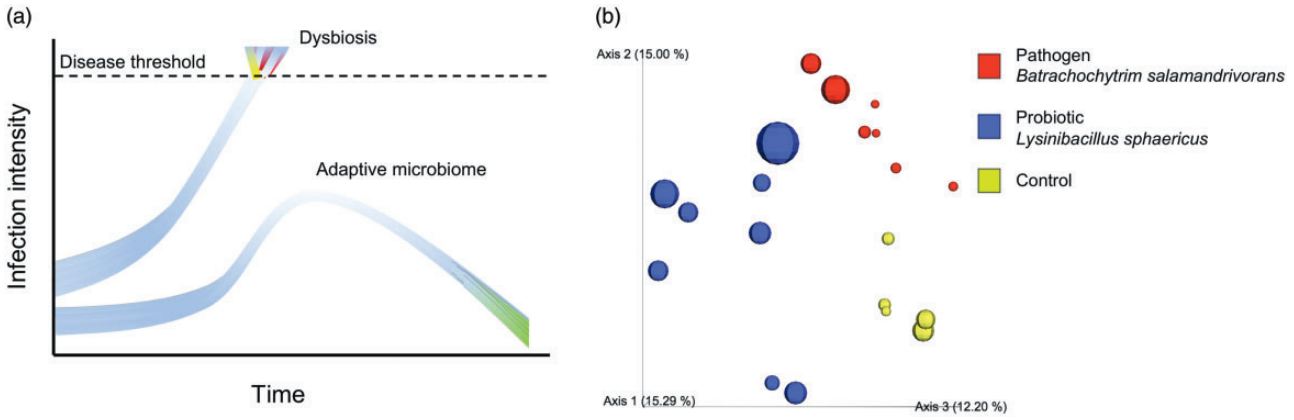

Figure 1. (a) Conceptual diagram of adaptive shifts in the microbiome acting as an extension of host immunity. The trajectory of infection is influenced by propagule pressure or infectious dose (initial timepoint), and may progress rapidly toward a disease threshold (dashed line), or may progress more slowly. In both cases, the microbiome can respond to infection with adaptive shifts in the microbiome including changes in both alpha-diversity (line thickness), or beta-diversity (line color), with a propensity for dominance of members that can compete with the pathogen. Upon passing the disease threshold, the microbiome may become dysbiotic. Alternatively, immune responses in combination with adaptive microbiome shifts can reduce infection intensity leading to host recovery. Manipulation of the microbiome can hasten this natural process and give the host an advantage over the pathogen. (b) Principal coordinates plot of larval spotted salamander (*Ambystoma maculatum*) skin bacterial communities showing long-term effects of exposure to a pathogen or probiotic. While hosts resisted infection to the skin pathogen *Batrachochytrium salamandrivorans* (red), a shift in the microbiome was evident 35 days post-exposure compared to controls (yellow). Similarly, the probiotic *Lysinibacillus sphaericus* did not permanently establish on the host, yet the trajectory of the microbiome was shifted on the developing hosts (blue). Points are scaled by predicted anti-*Batrachochytrium* function of the community based on Woodhams *et al.*[7] (antifungal function increased with probiotics, but was marginally significant; ANOVA, $F_{2,17} = 3.462$, $P = 0.055$). While there was a disruption of the microbiome, dysbiosis is not indicated because function and stability remained consistent despite the shift in community structure. Data from Barnhart[8] were re-analyzed with the QIIME2 pipeline[9] using Deblur to pick sOTUs[10] and unweighted UniFrac distance[11] was calculated for use in the principal coordinates analysis. (A color version of this figure is available in the online journal.)

prebiotics (nutrients that promote the growth of beneficial microorganisms) as supplements. This is because, as in human health, the use of pre- and probiotics for promoting animal growth or well-being generally have much less onerous regulatory burdens than products intended to treat infections or infectious disease. Therefore, much focus has been on the development of over-the-counter products that can be easily administered, and most (if not all) probiotics or microbial byproducts (e.g. enzymes) on the market currently fall within predetermined lists of safety, i.e. generally recognized as safe by the US Federal Drug Administration or qualified presumption of safety (QPS) by the European Food Safety Authority. As a result, the addition of claimed probiotics to livestock feed and pet food, referred to as direct-fed microbials, is becoming commonplace, although research data on this topic remain limited.[25,26]

The rising popularity of probiotics, even those whose safety and efficacy is not backed by scientific studies, is due to the fact that benefits are traditionally viewed as outweighing unknown side effects because treatment can result in myriad positive outcomes, although the mode of action is often unknown. They can relieve clinical symptoms such as diarrhea,[27–30] speed recovery after gastrointestinal disease,[31] and reduce or clear infection by specific pathogens. For example, gut colonization by socially acquired microbes has been shown to reduce parasite load in bumble bees,[32] and in honey bees, two strains of gut bacterial strains have been found to show probiotic effects against nosema infection.[33] Some probiotics have the added benefit of providing additional nutrients to the host or allowing for more efficient extraction of nutrients from food, resulting in better growth performance. In livestock, poultry, and aquaculture, antibiotics have been widely used to increase growth and feed efficiency and to prevent infections,[34] yet studies have shown that probiotics can result in similar outcomes in cattle,[35] pigs,[36,37] chickens,[38,39] and in aquaculture,[40,41] fueling the commercial development of probiotics as an alternative to antibiotics.[42,43] In addition to promoting healthy growth development without antibiotics, certain probiotics such as live yeast can help to stabilize gut pH, decreasing risk of acidosis, a common condition affecting ruminants.[44] Probiotics have also been used to help improve environment quality (e.g. water quality in aquaculture or domestic ponds) or reduce pathogens in the animal's environment, thereby reducing risk of acquiring infections. These practices are prevalent in aquaculture, a sector that has experienced major economic losses due to microbial infectious disease outbreaks and which also faces public pressure to reduce antibiotic use.[40,41]

Probiotic microbes can also play a key role in providing host immunity against pathogenic microorganisms by, for example, altering biofilms and epithelial turnover, and producing antimicrobial molecules.[45] Amphibians given a probiotic bath in local strains isolated from resistant amphibian species are better able to clear infection by the fungal skin pathogen *Batrachochytrium dendrobatidis* (*Bd*) and less likely to suffer mortality.[46–48] Studies show that strains common to amphibian skin such as *Janthinobacterium lividum*, *Serratia* spp., and *Pseudomonas* spp., produce small molecule antifungal compounds.[49,50] With the decline of hundreds of amphibian species linked to *Bd*,[51] efforts to treat these infections using probiotics are gaining increasing traction. Antifungal volatile organic compounds produced by microbes have been used to target infectious agents of

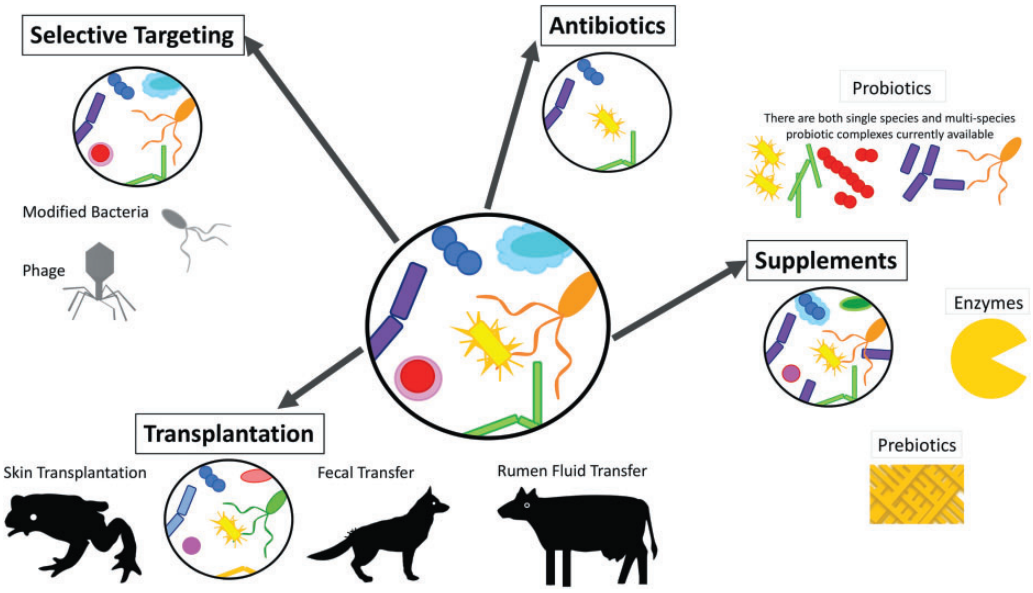

Figure 2. Overview of different modes of microbiome manipulation. Antibiotics can eliminate non-target organisms in the community in addition to the intended target. Supplementation involves addition of a single or combination of beneficial cultured strains into the community (probiotics), the addition of nutrients that promote the growth of beneficial microbes (prebiotics), or the addition of microbe-produced bioactive enzymes. Transplants add in an uncultured but live microbial consortium from donor hosts to alter the existing community in the recipient, and directed therapies like phage and gene editing can be effective ways to eliminate target organisms without altering the surrounding community. (A color version of this figure is available in the online journal.)

snakes and bats (e.g. White-nose fungus) as well,[52,53] further showing great potential of probiotics for aiding conservation of animals under threat from infectious disease. Perhaps the best-studied and most widely used probiotics are lactic acid bacteria (LAB; Figure 3) such as *Lactobacillus* spp., which are commensals in the gastrointestinal tract and now commonly associated with health benefits and fermented foods. It is thought that the ability of LAB to produce bacteriocins, a diverse group of antimicrobial compounds, aids in their ability to outcompete other bacteria (including pathogenic ones) for niche space as well as preventing their colonization.[59,60] For example, bioactive molecules produced by *Lactobacillus acidophilus* has been shown to help reduce pathogenesis caused by enterotoxigenic *Escherichia coli* in young pigs.[61]

Probiotic protection may additionally occur through activation of host immune responses, and research using teleost fish have significantly improved our understanding of the mechanisms of action of beneficial bacteria for host health, including effects on immune development, antibody production, mucosal homeostasis, and responses to stress.[45] For example, sphingolipids produced by the dominant commensal *Flectobacillus major* contribute to the mucosal immune system of rainbow trout, *Oncorhynchus mykiss,* by modulating mucosal and systemic immunoglobins and B cells.[62] Similar effects on immune regulation has been shown in chickens for example, where Lactobacillus species fed as DSMs results in both higher antibody production, and a reduction in enteric pathogens such as *E. coli* and *Salmonella*.[63,64]

Probiotics can also act beneficially by actively preventing colonization or proliferation of harmful microbes through competitive exclusion,[65] and ecological models suggest that if the addition of new microbial taxa to a system results in increased competition, it can have a stabilizing effect on the microbiome.[66] Indeed, this prediction is supported by some probiotic studies. For example, cultures from mucosal scrapings, but not luminal scrapings, significantly reduce *Salmonella* colonization in chickens, providing evidence that increasing competition for mucosal binding sites is one way to limit infection by this pathogen.[67] In plants, a probiotic cocktail applied to the phyllosphere is protective against infection by the foliar pathogen *Pseudomonas syringae*, but application of fertilizer abolishes the beneficial effect indicating that microbial competition for resources may be the mechanism leading to protection.[68]

The prophylactic properties of probiotics, especially multi-strain cocktails, most likely result from a combination of these modes. Unfortunately, the mechanism by which most probiotics work to protect the host remains unclear.[54] There is therefore a need for controlled studies where potential probiotic strains are not only isolated, cultured, and tested *in vivo* for effectiveness, but also experimentally tested to determine mode of action. It is also important to assess whether probiotics exhibit general effectiveness or specificity across animal hosts, and whether the mode of action is a constitutive property or context dependent (e.g. temperature, abundance, microbial community; see Woodhams *et al.*[69]). For some strains, highest efficacy appears to be achieved if the bacteria are from the same type of host or environment as the target host. For example, while many LAB can successfully adhere to intestinal mucus in a wide range of host species,[70] the commensal bacteria *Lactobacillus reuteri* shows high levels of host-specificity,[71] and can require matching of source and target hosts to ensure its effective colonization.[72] Similarly, the different needs across developmental stages

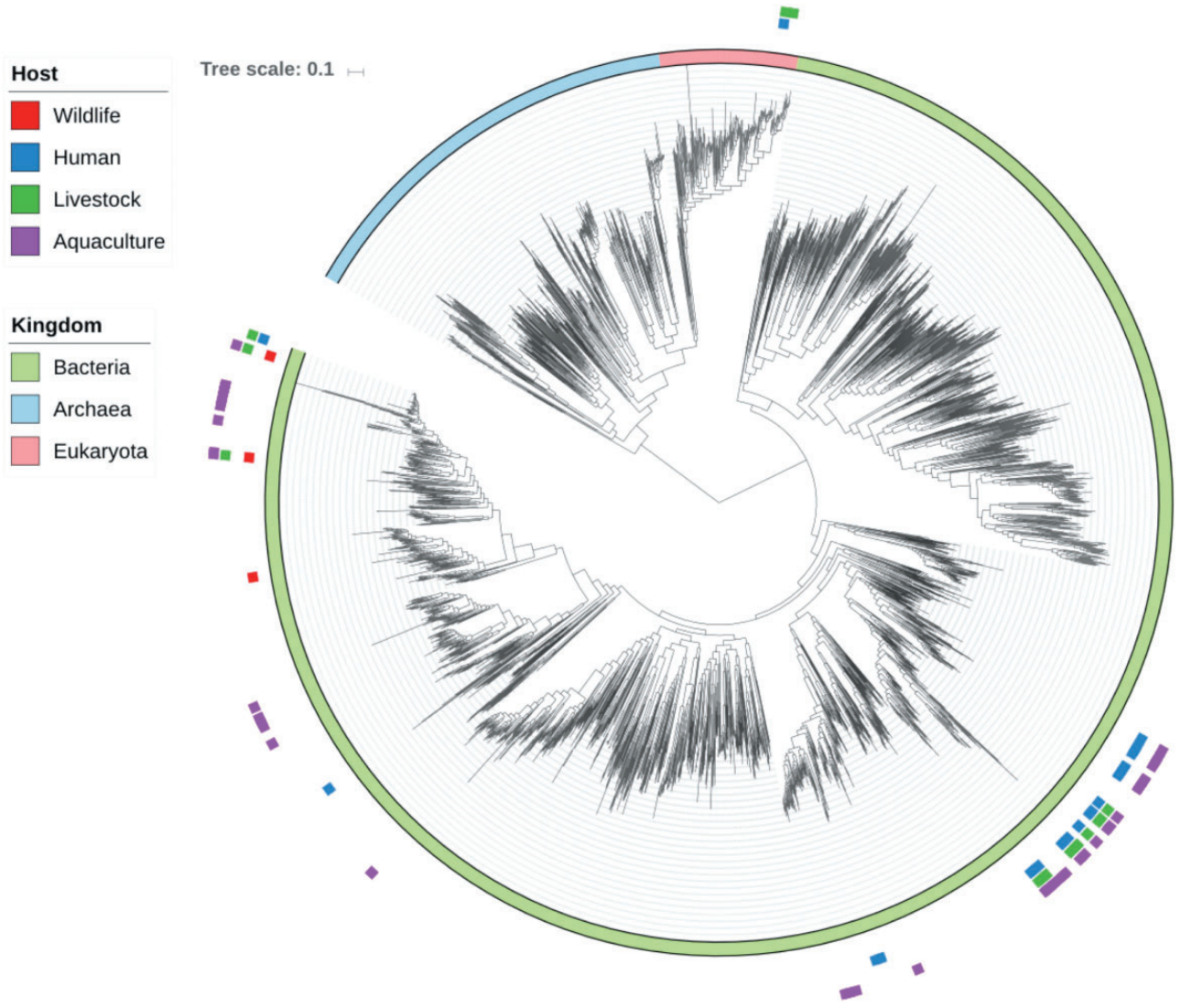

Figure 3. Microbial tree of life highlighting genera that have been tested as probiotics (direct-fed, enzyme production or forage additive). In total 37 microbial genera were identified from recent reviews[54–56] and annotated on the microbial phylogenetic tree[57] using iTOL.[58] They are primarily comprised by bacterial groups Bacillus and Lactobacillus (LAB), but also include Bifidobacterium, Enterococcus, Lactococcus (LAB), Megasphaera, Pediococcus (LAB), Propionibacterium, and fungal groups Aspergillus, Saccharomyces, and Schizosaccharomyces (Supplemental Tables 1 and 2). Boxes represent the host group(s) for which the probiotic is used colored by host type. The color bars represent the kingdom level taxonomy over all branch tips. (A color version of this figure is available in the online journal.)

should also be considered in probiotic development. For example, a calf's diet may require microbes able to utilize fiber whereas adult cows will need microbes able to utilize starch to allow for fast growth and production.

Community-level approach to reset the microbiome

Another approach to treating highly dysbiotic microbiomes is to introduce a new consortium of microbiota using transplantation from a healthy donor. Similar to probiotic cocktails, the idea is that the microbes in the consortium act synergistically to increase efficacy beyond what an individual microbe would provide. The notion of fecal microbiota transplants (FMT) is first mentioned in Chinese literature from the 4th century,[73] but there has been a recent resurgence of interest into the method due to its incredible efficacy in people whose microbiomes have become dysbiotic with recurrent *Clostridium difficile* overgrowth.[74,75] In animals, records of cud transplants in cattle used to treat digestive issues date back to the late 1700s,[76] and use of rumen "transfaunation" is common current practice.[77] For example, fluid from the rumen of fistulated healthy cows is frequently transplanted into individuals recovering from intestinal disruptions and abomasal displacements.[77] However, recent attempts at treatment show that efficacy of FMTs is limited and variable among conditions treated. In canine pups with parvovirus that underwent the procedure, faster resolution of diarrhea was observed; however, mortality rates were not significantly decreased.[78] Similarly inconclusive results were achieved in rhesus macaques with chronic diarrhea.[79] However, extensive work has been performed on manipulating the microbiome in chickens, and probiotics and/or

fecal transplant approaches have been shown to protect against a number of pathogenic species including *E. coli*, *Salmonella*, *Clostridium perfringens*, *Listeria* spp., and *Campylobacter* spp.[42,80–82] Fecal transplantation has also been shown to reduce morbidity and mortality associated with circovirus in pigs.[83]

Some studies suggest that variation in treatment success may be explained by variation in resilience of the host microbiome to manipulation. For example, while rumen fluid transfaunation has been shown to have a clinical benefit, it has been shown that microbiomes of healthy individuals are highly host-specific and resilient,[84] rapidly re-establishing following transplantation from another individual.[85] Similarly, colonization resistance not only prevented shifts in the skin microbiome of adult frogs upon attempting skin microbiota transplant, this resistance came at a cost to the hosts mounting immune defenses and reduced body mass.[86] Studies show that skin microbiome transplants in amphibians may have more effect during particular developmental windows when host immune defenses are more permissive of colonization (e.g. tadpole stage; see Davis *et al*.[87]). In most animal studies, it is unknown whether an experienced benefit is conferred via lasting microbiome change, and most studies to date have used small sample sizes and limited sampling timepoints, complicating interpretation relative to human and rodent studies of fecal or cecal transplantation.

Other hosts are not the only sources that have been investigated for potentially beneficial microbial consortia. Considering close ties between animals and their surrounding environments (e.g. see Hyde *et al*.[88]), it is not surprising that there is considerable interest in testing further whether ongoing input of environmentally derived microbes is essential for example, in amphibian health and *Bd* resistance.[89] In plants, the concept of soil microbiomes that are disease-suppressive has gained considerable currency,[90] and whether particular components of the soil microbiome such as non-tubercular mycobacteria confer disease-suppressive benefits to livestock or even humans remains an active topic of study.[91] Intriguingly, *Mycobacterium vaccae*, a soil bacterium, has immunomodulatory properties and is able to reverse deleterious phenotypic consequences associated with social stress in mice;[92] thus the potential for environmentally derived bacteria to modify physiological or even behavioral traits in animals warrants further investigation.

Selective targeting

Several different methods provide the ability to target a specific member of the microbiome, rather than altering the microbiome as a whole (although there is always the potential for cascading effects, especially if the single organism targeted is a keystone species for the microbial ecosystem). Phage therapy is the introduction of specific phage that targets an individual species or strain of bacteria. Phage therapy can be as effective as antibiotics in treating certain infections in livestock, and in preventing downstream effects in humans from consuming animal products contaminated by *Salmonella*, *Listeria*, *Campylobacter*, or *E. coli*.[93,94] Phage therapy directed against *Paenibacillus larvae* has also been effective against foulbrood in honeybees,[95] although trials have had mixed success.[96,97] Gene editing via CRISPR-Cas, in which a specific gene in a specific strain is targeted, is an emerging technology with many areas of possible application in animal health.[98] For example, the specific nature of the CRISPR-Cas system allows for its potential use as very efficient antimicrobials through self-targeted destruction of the genome or deletion of genomic regions that encode for virulence factors.[99] Bacterial secretion systems, some of which (type 3, 4 and 6 secretion) are able to deliver proteins directly across the host cell membrane into target cells, offer additional prospects, as these systems can be hijacked for delivery of proteins such as vaccines and therapeutics or inducing targeted cell death.[100] Pyocins, phage tail-like structures that kill bacteria by punching a hole through the cell membrane,[101] also hold considerable promise for precise editing of microbiomes to remove particular species or strains. In general, there are many prospects for engineered or modified bacteria and phages that are able to kill pathogenic bacteria, act as gene transfer agents to confer beneficial functions, or transfer proteins into host cells that confer benefits. However, there are still many technical and biological challenges and risks to overcome with these targeted methods and established use in animal applications are likely far in the future. For example, as with antibiotics, bacterial resistance against phages used in phage therapy can evolve rapidly,[102] and CRISPR-Cas nucleases can cause non-target mutations in the genome,[103] potentially altering interactions with unknown consequences (although methods reducing these mutations have recently been developed[104]).

Future directions and conclusion

The studies and techniques highlighted here show that manipulations of the microbiome can be used to positively affect animal health. However, several large unknowns still exist. First, the time frame of treatment efficacy can vary significantly among approaches. Some treatments only need to be applied once, whereas others must be applied repeatedly, and in general the rules for predicting a treatment schedule for a microbiome-directed therapy are unknown because the evidence base is insufficient. The treatment schedule may depend on the mechanism: for example, antimicrobial production by a high-dose probiotic may keep pathogen levels low enough until the host can mount a sufficiently strong immune response to clear the pathogen, in which case the treatment may not need to be repeated. However, at lower doses, the probiotic may simply keep the pathogen from increasing in abundance further, and if the probiotic exerts its effect while passing through the gut rather than establishing,[105,106] continuous administration may be required. Similarly, in humans with *C. difficile* associated disease, the gut microbiomes of patients receiving an FMT all diverged away from resembling the donor over time, but stayed within the range of variation of other healthy adults,[107] suggesting that for this condition shifting the gut away from a dysbiotic state is

enough to prevent recurrence. Yet, other indications such as IBD[108] and autism[109] have required repeated FMT rather than a single transplant.

Second, the efficacy of particular methods may depend on the animal's developmental stage, especially early in life when both the microbiome and immune system are changing rapidly. For example, we noted above that skin microbial transplants may be most effective in protecting amphibians against *Bd* when applied at the tadpole stage. For most animals, it is still unknown whether particular life stages exist where bioengineering of the microbiome is most beneficial. For example, studies in humans and mice suggest that early exposures to a wide variety of microbes, both from conspecifics and from the environment, help better train the immune system to fight off infections later in life (e.g. see Stein *et al*.[110]). At the same time, variability among timepoints in infant microbiomes is very high,[111,112] and effects from defined interventions may be transient in the face of this variability. Both of these points may well be true in other animals. More studies are needed to determine whether and which microbiome-directed impacts early in life have lasting effects, and to identify optimal developmental windows of opportunity, with the recognition that these could vary among animal species.

Third, probiotics in particular requires that the probiotic strains can be isolated, cultured, and brought to the animal to be treated in a stable form. To identify fundamentally new probiotics, rather than variations on a well-worn theme, there is a clear need for high-throughput culturing of a broad phylogenetic range of microbes from a diverse array of animal hosts to overcome the current bias of cultured microbes from humans or model systems. Of all known microbial genera[57] only 37 are represented in current probiotic products[54–56] (Figure 3). Most efforts have been aimed at bacteria, as reflected by published research; for example, only 19% of published studies focus on fungal agents (compared to 79% on bacteria).[54] Expanding further into these other domains could vastly increase our microbial toolkit (e.g. see Kearns *et al*.[113]). Expanding studies beyond traditional models can also be very important: for example, hamsters are a much better model than mice for *C. difficile* infection,[114] and ferrets are an excellent model for influenza research,[115] due to details of their biology that happen to resemble humans more than other mammals to which they (and we) are more closely related. What excellent animal models for infectious or microbiome-linked diseases might lie undiscovered in zoos or rainforests? Relatedly, a recent study noted that up to 80% of unique microbial marker sequences from lemur GIs were from unidentified bacteria (based on 97% sequence identity to a reference database).[116] Other under-studied animal species could be equally rich sources of potentially novel microbes, and microbiome studies can help identify such key microbial sources that should be targeted for conservation. An assessment of the probiotic literature discovered that less than 2% of all studies target animals of conservation concern, compared to 66% for animals of agricultural concern (aquaculture, livestock and poultry, and crops).[54] Consequently, opportunities for probiotic discovery and application of microbiome-targeted therapies in conservation are also still in their infancy, yet hold immense potential. We have already highlighted how probiotic research of *Bd*-resistant strains could help combat worldwide amphibian declines. Other research has emphasized how microbiome research could help monitor health, movements, and disease transmission in wild populations,[117] as well as inform captive breeding programs[118] and management including re-introduction efforts from captive populations into the wild.[119,120] The field of conservation biology is therefore poised to benefit greatly by increased focus on the microbiomes of both wild and captive species.

Lastly, all aspects of microbiome-directed therapies in all animal species, including our own, could benefit from more rigorous experimental scrutiny. Both benefits and potential side effects must be quantified in double-blind placebo-controlled studies (animals are sensitive to expectations of their handlers), particularly when large quantities of bacterial cultures and/or their bioproducts might end up in the environment. Mechanistic studies that reveal not only whether a particular formulation alleviates symptoms but that allow us to understand the mechanism of protection are also needed, especially as we seek to generalize what we know from a handful of probiotic taxa and therapeutic strategies to the rest of the host-associated microbial world. In this vein, organoids (stem cell-derived structures that mimic real organs) may offer exciting avenues to study species- and host-specific interactions between tissues and microbes, including the many species of animals that it would be impractical to keep and breed in the laboratory. Finally, we still need to fully understand the healthy range of interindividual, temporal and spatial variation to most effectively use the microbiome as a screening tool for host health, and to define and treat states of dysbiosis. However, as in human health, applications of the microbiome in animal health are expanding rapidly, and the prospects for application in domestic pets, farm animals, and for conservation are exciting.

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DECLARATION OF CONFLICTING INTERESTS

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