Chapter 23
Defensive Microbiomes: A Widespread Phenomenon in Nature

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Abstract Microbes, such as bacteria and fungi, produce antibiotic compounds during competition with other species for resources such as space and nutrients. Such compounds have underpinned much of modern medicine as, in their purified form, they are widely prescribed by humans as antibiotics to cure bacterial and fungal infections. However, numerous other organisms have been using the antimicrobial products of microbes to protect themselves against disease for millennia, with many eukaryotic species forming close mutualistic interactions with defensive microbes which live on or within their host species. In addition to producing antibiotics, these microbes can inhibit infection by stimulating their host's immune system and by competing with, and thus excluding, pathogenic organisms. Developing an understanding of how interactions between hosts and protective microbes arise and are effectively maintained over time, despite the evolution of pathogenic resistance, could inform our own use of antibiotics as well as novel therapies. This essay will discuss the prevalence of defensive microbiomes in nature and how their assembly may inform future strategies to protect against disease.

23.1 Microbes Underpinning Modern Medicine

Humans have been widely using antibiotics to cure diseases caused by bacterial and fungal agents since the late nineteenth century. Almost all of the earliest antibiotics, and many of those used today, are the purified natural products of microbes which have been isolated from environmental samples, such as soil (Gould 2016; Hopwood 2007). In natural systems, microorganisms produce these antibiotic compounds to kill or inhibit the growth of other microbial species during competition for resources such as nutrients or space.

Even before it was known that microbes were the source of many antimicrobial compounds, ancient civilisations are thought to have used their products to prevent

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and cure diseases. For example, there is evidence that societies in Egypt and Rome topically applied mouldy bread to wounds to prevent infections. Presumably, the fungal species that were growing on the bread were producing antimicrobials that inhibited the proliferation of pathogenic microbes (Gould 2016). Famously, Alexander Fleming serendipitously showed that fungi could make such compounds by leaving agar plates growing a bacteria, called *Staphylococcus,* uncovered whilst away on holiday. He noticed that a contaminating fungus, called *Penicillium notatum,* created bacteria-free zones wherever it was growing, suggesting that the fungus was producing a potent antibacterial which he named penicillin (Fleming 1929). Penicillin was the first modern antibiotic to be mass-produced and was hugely important during World War II when it was used to treat the infected wounds of injured soldiers (Dias et al. 2012). Around the same time that Fleming discovered penicillin, a scientist called Selman Waksman was studying a phylum of bacteria called Actinobacteria. Waksman observed that many actinobacterial species isolated from soil could selectively inhibit the growth of other microorganisms when they were grown together in the laboratory (Hopwood 2007). This finding led to a systematic search for actinobacterial isolates that could kill disease-causing bacteria and fungi. This, in turn, resulted in the isolation of several clinically useful antibiotics, including streptomycin which is produced by the bacterial species *Streptomyces griseus* and can be used to treat a variety of bacterial infections, including tuberculosis (Schatz et al. 1944). Currently, the phylum Actinobacteria is responsible for producing over half of all clinically useful antibiotics (Hopwood 2007; van der Meij et al. 2017; Devine et al. 2017). Genome sequencing has also revealed that many isolates have the genetic potential to produce a huge diversity of different natural products that may demonstrate novel activities and could also be exploited in the future.

Thus, microbially produced antibiotics have been instrumental in reducing human mortality throughout history, but particularly over the last century when they became widely prescribed in the clinic. However, it is not only humans that have exploited the products of microbial competition to prevent infection and disease. In fact, an increasing number of organisms with markedly distinct natural histories are being found to accumulate microbial species that produce antimicrobial compounds. These microbes, in turn, are being shown to protect their hosts against infections caused by parasitic and pathogenic microorganisms.

23.2 The Microbiome

Almost all organisms, at some stage during their lifecycle, interact extensively with a complex community of microorganisms which make use of host resources and are acquired from the host's environment. These diverse microbial assemblages, their collective genomes as well as the host habitat, are collectively referred to as an organism's microbiome (Marchesi and Ravel 2015). Advances in nucleic acid sequencing technologies have enabled us to investigate the composition and function

of these microbial communities in great detail and many studies have demonstrated that there are often consistent patterns in the microbial groups that associate with a particular host species. For example, in humans the early-life infant gut is almost always dominated by certain species of the bacterial genus *Bifidobacterium*, which are then superseded by members of the phyla Firmicutes and Bacteriodetes later in adult life (Matamoros et al. 2013). Furthermore, several microbial communities, for example those associated with plants and insects, have been found to be tightly linked to host phylogeny, suggesting that particular microbial assemblages are maintained and are changing over evolutionary time alongside their host (Brucker and Bordenstein 2012; Fitzpatrick et al. 2018; Sanders et al. 2014).

The non-random accumulation of microbial communities within the host microbiome suggests that host species may be able to influence which microorganisms they associate with. In fact, hosts are expected to experience strong natural selection to filter the enormous pool of microbial species available to them and evolve mechanisms that encourage the persistence of microbes that provide them with significant fitness benefits (Archetti et al. 2011; Foster et al. 2017; Scheuring and Yu 2012). Microorganisms can be advantageous to their host in a number of different ways. For example, many provide their hosts with nutritional benefits by breaking down complex, otherwise indigestible molecules, or by supplementing the host diet with essential nutrients through pathways such as nitrogen fixation or phosphate solubilisation. Other symbionts, which are the focus of this essay, can provide protective benefits to their host by inhibiting the growth and invasion of pathogenic and parasitic organisms.

23.2.1 Leafcutter Ants and Their Protective Microbes

Attine leafcutter ants represent a fascinating example of a defensive mutualism between antimicrobial-producing bacteria and a eukaryotic host. Leafcutter ants are indigenous to Central and Southern America and are renowned for their specialised agricultural activities. Worker ants collect fresh leaf material from their surrounding environment which is then taken back to their nests and used as a compost to grow a mutualistic food fungus, called *Leucoagaricus gongylophorous* (Worsley et al. 2018; Currie 2001). In return for a growth substrate, the fungus produces swellings called gongylidia which are rich in lipids and proteins. These are harvested by the ants and used as the sole nutrients source for the queen and her larvae (De Fine Licht et al. 2014; Currie 2001).

Although this system is effective, the clonal fungal cultivar is a rich food source and is therefore at risk from being parasitised by other organisms. Indeed, another fungus called *Escovopsis weberi* is highly specialised to grow on the food fungus and, if left unchecked, can cause ant colony collapse. This occurs when the ants are starved of their food source and eventually die or abandon their nest (Currie et al. 1999a; de Man et al. 2016). However, the ants have evolved several lines of defence against such invasions, including specialised behaviours that enable them to detect

and weed out pieces of infected garden (Currie and Stuart 2001). As an additional line of defence, leafcutter ants also interact extensively with antibiotic-producing bacteria in the phylum Actinobacteria. These bacteria grow as a visible white mass on specialised structures that are found on particular regions of the ants cuticle (Currie et al. 1999b; Andersen et al. 2013; Kost et al. 2007; Currie et al. 2006). Fueled by competition for the nutrients provided by the ant host, the bacterial mutualists produce a variety of antibacterial and antifungal compounds. These compounds have been shown to inhibit the growth of other microorganisms that might invade the fungus garden, including the parasite *E. weberi,* and have also been identified at active concentrations in the nests of leafcutter ants (Currie et al. 1999b, 2003; Barke et al. 2010; Haeder et al. 2009; Sen et al. 2009; Worsley et al. 2018; Schoenian et al. 2011).

However, there is an interesting evolutionary twist to this story—*E. weberi* has recently been shown to combat both of the ants two major lines of defence. The parasite has evolved to produce chemicals, called shearinine D and melinacidin IV, that not only prevent the weeding behaviours of the ants by causing paralysis and eventual mortality, but that also inhibit the growth of the actinobacterial symbionts (Heine et al. 2018). Despite this, widespread resistance does not seem to be the case in nature and the attine-actinobacteria mutualism is thought to have survived and enabled ants to farm their fungus for over 50 millions years (Currie et al. 2006). Instead, a constant coevolutionary arms race seems to be occurring within the leafcutter ant system, involving the *Escovopsis* parasite, the ants, and their protective symbionts. The evolution of resistance in *Escovopsis* drives the evolution of novel antimicrobial compounds in the mutualistic Actinobacteria and therefore prevents the dominance of resistant parasite strains (Currie et al. 2006; Pathak et al. 2019; Worsley et al. 2018). Additionally, different actinobacterial species on the ants' cuticle produce different types of antimicrobial compound, resulting in a form of multidrug therapy whereby parasites are faced with too many compounds to evolve resistance to all of them at once (Barke et al. 2010; Seipke et al. 2011).

23.2.2 Actinobacteria as Protective Symbionts

Leafcutter ants are not alone in recruiting antibiotic-producing Actinobacteria to protect against disease. In fact, Actinobacteria are thought to be involved in approximately half of all described examples of defensive mutualism (Kaltenpoth 2009) and interact with a range of terrestrial and marine invertebrates, as well as several plant host species (Kaltenpoth 2009; Seipke et al. 2012; Viaene et al. 2016). Apart from having a diverse secondary metabolism capable of producing many antimicrobial natural products, Actinobacteria are also characterised by a lifecycle involving filamentous growth and spore-forming stages. Members of this phylum are also capable of subsisting on a wide range of carbon sources and metabolic waste products that are often present at very low concentrations. Together, these characteristics may have enabled Actinobacteria to interact with a diverse range of hosts

and become so widespread as defensive symbionts (Kaltenpoth 2009). It is possible that many actinobacterial species began as commensals or mild parasites, competing with other microbial species for host resources. The production of antimicrobials during this microbial warfare may have, in turn, become beneficial to the host by preventing pathogenic infection. This may have driven the evolution of host mechanisms to ensure that Actinobacteria were consistently able to colonise the microbiome (Kaltenpoth 2009). The spore-forming capabilities of Actinobacterial species may have also aided this process by enabling species to resist environmental stressors that may be experienced in the absence of the host during inter-individual and inter-generational transmission (Kaltenpoth 2009).

The spore-forming capabilities of actinobacterial species may be particularly important during symbiosis with solitary 'Beewolf' digger wasps. Female solitary digger wasps (in the genera *Pilanthus*, *Trachypus* and *Philanthinus*) lay their eggs in burrows, which they dig into the soil and provision with a paralysed honey bee; the developing larvae feed on the bee before spinning cocoons (Kaltenpoth 2009; Kaltenpoth et al. 2005). The brood chambers are humid and damp providing optimal growth conditions for a variety of fungi and bacteria. However, to prevent developing infections, the mother wasp coats the brood chamber walls with secretions containing a species of Actinobacteria, called *Candidatus Streptomyces philanthi,* which the wasp cultures in specialised antennal glands (Kaltenpoth et al. 2005, 2006). These bacteria then become incorporated into the larval cocoon and produce antibiotics on the cocoon surface (Kroiss et al. 2010). Remarkably, the *Streptomyces* symbiont can remain viable on the cocoon wall as spores for up to nine months before the larva emerges, despite the cocoon surface being a very poor environment with limited nutrients availability (Kaltenpoth et al. 2010). This long-term survival also allows the *Streptomyces* bacteria to be vertically transmitted across wasp generations as they are then taken-up by the fully-developed females that emerge from the cocoons (Kaltenpoth 2009; Kaltenpoth et al. 2010).

23.2.3 Competitive Exclusion and Modulation of the Host Immune System

Actinobacteria are important symbionts for many organisms, however, defensive microbes are not limited to this bacterial phylum. In fact, a large number of other bacteria, across a wide range of phyla, are also known to provide their hosts with protection against disease. For example, hoopoe birds (*Upupa epops*) are known to culture high densities of *Enterococcus* bacteria in their uropygial (preen) glands; these bacteria produce volatile antimicrobial substances that are known to inhibit the growth of feather-degrading microorganisms (Martin-Platero et al. 2006; Martin-Vivaldi et al. 2010). Similarly, embryos of several species of crustacean are coated in a dense growth of Gram-negative bacteria that produce antifungal compounds

against the fungal pathogen *Lagenindium callinectes* (Gil-Turnes and Fenical 1992; Gil-Turnes et al. 1989).

Most described examples of defensive mutualisms involve bacterial partners. In comparison, far less is known about the role that fungal species can play in providing protective benefits to host organisms. This is partly because, relative to bacteria, fungi remain hugely understudied in the context of the microbiome. They can also be difficult to culture, making it hard to characterise their function. However, genomic studies as well as bioactivity assays using fungal isolates have demonstrated that many fungal species encode a diverse secondary metabolism capable of making a large arsenal of different antimicrobials (Rateb and Ebel 2011). Fungal species are also hugely abundant in the microbiomes of many species and in some cases, such as sponges, are transmitted maternally across generations suggesting that they can be closely associated with their host organisms (Maldonado et al. 2005). One of the few examples of a defensive fungal symbiont is the association between leaf rolling weevils (*Euops chinensis*) and the fungal species *Penicillium herquei*. The *Penicillium* symbiont is added to the leaves in which the weevils roll their eggs and larvae. Here, it has been shown to produce the antimicrobial scleroderolide which inhibits the growth of microbial pathogens (Wang et al. 2015). With greater study and the development of new techniques, further examples of defensive partnerships involving fungi may come to light.

Antibiotic production is a key mechanism by which microbes are able to protect their host against infection. However, this often also works in addition to, or in combination with, other mechanisms. By colonising a host and taking up resources such as space and nutrients, symbiotic microbes can also competitively exclude pathogenic or parasitic microorganisms which use the same resources. For example, in mice, a bacterial species called *Bacteroides thetaiotaomicron*, is known to consume carbohydrates that are required for the growth of *Citrobacter rodentium* (a mucosal pathogen of mice). By preventing access to a key resource, *B. thetaiotaomicron* is able to exclude the pathogenic species from the mouse intestinal lumen (Buffie and Pamer 2013).

Members of the microbiome can additionally contribute to the defence of their host by priming the host immune system so that it can efficiently respond to pathogenic attack. For example, evidence from mouse models suggests that commensal bacteria in the intestinal tract can enhance host immunity by directing the development of immune cell populations involved in both innate and adaptive immune processes. These bacteria also promote the production of antimicrobials and pro-inflammatory factors by cells in the gut (Buffie and Pamer 2013; Hooper et al. 2012). Similarly, bacterial symbionts that colonise plant roots, such as *Bacillus*, *Streptomyces* and *Pseudomonas* species, have also been shown to prime the plant immune system, resulting in an elevated and accelerated response to pathogenic infection (Pieterse et al. 2014; Kurth et al. 2014). The plant host recognises residues on the surface of these beneficial microbial species (called microbial associated molecular patterns, or MAMPs) which leads to the activation of signaling cascades involved in mounting an immune response. These pathways are then primed to respond to pathogenic invasion (Pieterse et al. 2014; Selosse et al. 2014).

23.3 Selecting Protective Microbes

In many cases, protective microorganisms appear to be a consistent part of, or even dominate, the host microbiome. For example, Actinobacteria massively outnumber other microbial species on the cuticles of leafcutter ants (Andersen et al. 2013) and Bifidobacteria dominate the infant human gut, where they inhibit the growth of pathogens and promote immune system development (Matamoros et al. 2013). A great challenge is to understand how a host can selectively associate with these microbial species when it is exposed to a huge environmental pool of microbes. Such knowledge could enable us to enhance the presence of beneficial microbes, for example in the human gut following a course of antibiotics, or in the roots of economically important crop plant species to reduce yield losses caused by disease.

23.3.1 The 'Partner Choice Problem'

The issue of how a host recruits specific microbes is often referred to as the 'partner choice problem'. This is because for a host to be able to accumulate beneficial species, it must be able to distinguish between different strains in its environment and limit its interactions to microbes that provide it with significant benefits (Archetti et al. 2011). For many instances of partner choice in nature individuals can distinguish between better or worse partners via costly phenotypes. For example, elaborate male ornaments, such as tail feathers, facilitate mate choice in many bird species, since only high-quality individuals can afford to invest in these (Archetti et al. 2011). However, with microbial-host interactions, it seems unlikely that such signals could exist and be detected by the host, allowing discrimination between thousands of microbial species. Instead other mechanisms are thought to enable hosts to indirectly bias the accumulation of beneficial microbial species from their environment (Archetti et al. 2011; Boza et al. 2019; Scheuring and Yu 2012).

One mechanism by which a host could encourage the colonisation of protective species within its microbiome is by giving certain microbes preferential access to resources in the host niche. The simplest way by which this can occur is by transmitting them vertically across host generations, rather than acquiring them horizontally from the environment (Boza et al. 2019). This is the case for leafcutter ants, which remain sterile before they hatch from the pupal stage (Marsh et al. 2014). Following hatching, they are inoculated with the antibiotic-producing Actinobacteria that grow on older worker ants, within a 24 h window (Marsh et al. 2014). These filamentous Actinobacteria then bloom over the ant cuticle in the absence of any competition, before receding to grow around specialised crypts which are thought to supply the cuticular microbiome with resources (Currie et al. 2006).

A second mechanism by which a host could drive the accumulation of beneficial microbial species is by providing its microbiome with specific nutrients that are

preferentially utilised by microorganisms with the desired metabolic capabilities, such as antibiotic production (Boza et al. 2019; Foster et al. 2017). This hypothesis can be extended, since resources can also drive competition between strains. Therefore, hosts could also provide their microbiome with resources that ensure beneficial microorganisms successfully outcompete other species (Archetti et al. 2011; Scheuring and Yu 2012). There are several examples of this occurring in corals which, along with their dinoflagellate symbionts, produce large quantities of the compound dimethylsulfoniopropionate (DMSP) (Raina et al. 2013). It is thought that bacteria that can degrade DMSP may have a nutritional advantage over non-degraders and that this compound could therefore be important in structuring the initial coral microbiome (Apprill et al. 2009; Raina et al. 2010). Interestingly, one bacterial coloniser, called *Pseudovibrio*, can also use DMSP as a precursor to produce antimicrobial compounds that inhibit the growth of coral pathogens, suggesting that DMSP may also play a role in fuelling competitive exclusion and host protection (Raina et al. 2016).

Finally, a host can also direct microbiome establishment by producing compounds or barriers that block the colonisation and survival of non- beneficial species, whilst still enabling or promoting colonisation by beneficial species (Boza et al. 2019). Plants exude a variety of toxic molecules, called allelochemicals, which inhibit a broad range of bacteria, fungi and invertebrates, as well as other plants growing in close proximity (Neal et al. 2012; Hartmann et al. 2009; Bais et al. 2006). Beneficial microbial species must be able to tolerate allelochemicals to colonise the root microbiome of the host plant. The compound DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is an antimicrobial allelochemical that is constitutively produced by maize seedlings and is toxic to many bacterial species (Neal et al. 2012). However, the plant-beneficial species, *Pseudomonas putida*, is able to degrade DIMBOA and additionally upregulates the production of a broad-spectrum antibiotic called phenazine in response to detecting the compound, allowing effective colonisation of the root microbiome and host protection against fungal pathogens (Neal et al. 2012).

23.4 Taking Inspiration from Defensive Microbiomes

As discussed, defensive microbiomes appear to be a widespread phenomenon in nature and in several instances, such as in the leafcutter ant and beewolf digger wasp systems, there is evidence to suggest that they have remained effective at suppressing pathogens over millions of years. On the flip-side, humans have been extensively using antimicrobials as medicine and in agriculture for around a century, but we have seen a rapid rise in pathogenic resistance and a concurrent decrease in the effectiveness of clinically available antibiotics. This contrast begs the question of whether there is anything to be learnt from the protective mutualisms that have evolved in nature between hosts and microbial species and if an understanding of how protective microbiomes evolve could lead to new ways to combat infections.

23.4.1 Novel Antimicrobial Compounds

With the rapid emergence of drug-resistant pathogens, there has been a renewed effort to search for novel antimicrobials in the environment. In the past, antimicrobials were isolated from soil samples, however, frequent re-discovery of the same compounds has encouraged scientists to explore other niches for new antimicrobials. Increasingly, it is thought that defensive microbiomes may yield structurally diverse and novel compounds that have a greater efficacy against human pathogens (Adnani et al. 2017; Chevrette et al. 2019; Seipke et al. 2012). Within the microbiome, microbial species face intense competition for host resources. This fuels the production of antimicrobial agents as species produce them to inhibit the growth of competing microorganisms. Symbiotic species that rely on their host for resources must also ensure continued host survival. Thus, coevolutionary dynamics between invading pathogens, symbionts and host organisms, are expected to result in the continued evolution of novel antimicrobial compounds with distinct activities. These may be able to target pathogen populations that are clinically relevant (Pathak et al. 2019; Adnani et al. 2017; Chevrette et al. 2019). For example, a group of compounds called the formicamycins have recently been isolated from a species of *Streptomyces* growing in association with African *tetraponera* plant-ants; these compounds were shown to inhibit multidrug resistant pathogens which showed no evidence of being able to evolve resistance, suggesting that the formicamycins had a highly effective mode of action (Qin et al. 2017). Looking within microbiomes for new antimicrobials may also prove advantageous, since compounds produced by beneficial microbes that interact with a eukaryotic host are likely to prove less toxic to human cells, or those of other animal and crop species (Adnani et al. 2017).

23.4.2 Safeguarding Our Antimicrobials

There is evidence to suggest that several defensive mutualisms have remained effective for millions of years, with little evidence of pathogenic resistance evolving in these systems. For example, Beewolf digger wasps are thought to have been using the same antibiotics (produced by their *Streptomyces* symbionts) since the Cretaceous period (Engl et al. 2018). It is thought that pathogen resistance is avoided in this system because the *Streptomyces* symbionts produce a large variety of antimicrobials at any one time. In fact, it has been shown that multiple antimicrobials, that vary slightly in their structure and activity, can be produced from the same gene in the *Streptomyces* symbiont's genome (Engl et al. 2018). This variable cocktail, in addition to the targeted application of antibiotics in the larval brood chamber, is thought to reduce the chances of pathogen resistance evolving, as multiple cellular processes are targeted at once. Such multidrug strategies are thought to be common across vertebrate and invertebrate species (Florez et al. 2015). Leafcutter ants are also known to use multidrug therapy to combat infections in their fungal gardens;

worker ants host slightly different actinobacterial communities on their cuticles and each bacterial specie is capable of making multiple different antimicrobials, which are also thought to be constantly evolving (Barke et al. 2010; Seipke et al. 2011; Worsley et al. 2018). Although the use of multidrug therapy in humans is controversial, varying our antibiotic usage and ensuring that their application is targeted (to bacterial or fungal infections only), may help to safeguard novel antibiotics into the future.

23.4.3 Manipulating Microbiomes

Developing an understanding of the host mechanisms and environmental factors that influence the assembly of a microbiome could inform strategies to manipulate their composition. For example, there is great interest in being able to enhance the presence of beneficial, protective microbial species within the microbiome to improve or restore host health. An increasing number of studies are demonstrating that the human infant gut microbiome can be profoundly disrupted by factors such as antibiotic treatment, birthing method (C-section versus vaginal birth) and formula feeding (Mueller et al. 2015; Tamburini et al. 2016). This in turn is linked to an increased risk of developing infections caused by pathogenic strains, such as *Clostridium difficile*, as well as immune and metabolic diseases later in life (Mueller et al. 2015; Tamburini et al. 2016; Matamoros et al. 2013). Thus, scientists are investigating ways to restore the healthy infant gut microbiota and increase its resilience to infection. It is known that human breast milk contains a high density of complex oligosaccharides and long-chain polyunsaturated fatty acids and that each of these is preferentially consumed by a single species of co-adapted gut bacteria (Tamburini et al. 2016; Matamoros et al. 2013; Zivkovic et al. 2011). For example, specific oligosaccharides are known to promote the proliferation of Bifidobacterium species which play an important role in inhibiting the growth of pathogens and directing immune system development (Tamburini et al. 2016; Matamoros et al. 2013; Zivkovic et al. 2011). Many public health organisations now recommend breast feeding over formula milk whenever possible. Several studies have also looked into whether formula milk could be modified to include important components of breast milk; these would act as prebiotics to restore or promote the defensive microbiome (Borewicz et al. 2019; Mueller et al. 2015; Tamburini et al. 2016).

Similarly, there is a lot of interest in manipulating the root microbiome of key food crop plant species to suppress disease and improve harvestable yields (Newitt et al. 2019; Zhang et al. 2015; Ryan et al. 2009). This method could act as a potential alternative to the application of environmentally damaging chemical pesticides and also provide a mechanism of protection when there are no resistance genes available to breed into the crop of interest (Newitt et al. 2019). Several potential methods to manipulate plant root microbiome composition are beginning to be explored. This includes the application of antibiotic-producing biocontrol agents as probiotic seed coatings before sewing the crop (O'Callaghan 2016); this ensures that beneficial

strains are delivered to the soil directly surrounding the germinating seed, enhancing their potential to colonise and compete on the emerging roots. This strategy mimics the vertical transmission of strains seen by other eukaryotic hosts, such as leafcutter ants. Plants also release approximately 20% of the carbon that they fix during photosynthesis into the soil via their roots (Bais et al. 2006; Chaparro et al. 2013). This root exudate contains a huge variety of carbohydrates, proteins and organic acids which can all act as substrates or inhibitors for different microbial species (Bais et al. 2006). For example, secretion of the tricarboxylic acid intermediate, malic acid, by the plant species *Arabidopsis thaliana* has been shown to enhance the recruitment of the bacterial species *Bacillus subtilis* to roots when plants are infected with a foliar pathogen (Rudrappa et al. 2008). An increased release of malic acid from the roots initiates the movement of *B. subtilis* towards *A. thaliana* and promotes the subsequent formation of biofilms by this bacterial species on the plant roots (Rudrappa et al. 2008). In turn, *B. subtilis* is capable of priming the plant host's immune system, reducing the severity of infections (Rudrappa et al. 2008). Understanding the role of individual root exudates, like malic acid, as well as the genetics underlying their production and release from roots could enable crop breeders to create new plant lines that are more effective at attracting protective bacteria (Zhang et al. 2015; Ryan et al. 2009).

Thus, understanding how organisms interact with protective microbes in natural systems could provide the inspiration to develop novel methods of disease control for the benefit of human health, agriculture and conservation. Major challenges include unpicking the complexity of microbiomes and understanding the factors that contribute to microbial community variation and stability over time. New technologies could further enable the exploration of defensive mutualisms involving underexplored microbes, such as fungi and viruses, which could also open up novel avenues for disease prevention into the future.

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References

Adnani N, Rajski SR, Bugni TS (2017) Symbiosis-inspired approaches to antibiotic discovery. Nat Prod Rep 34(7):784–814. https://doi.org/10.1039/c7np00009j

Andersen SB, Hansen LH, Sapountzis P, Sorensen SJ, Boomsma JJ (2013) Specificity and stability of the *Acromyrmex-Pseudonocardia* symbiosis. Mol Ecol 22(16):4307–4321. https://doi.org/10.1111/mec.12380

Apprill A, Marlow HQ, Martindale MQ, Rappe MS (2009) The onset of microbial associations in the coral *Pocillopora meandrina*. ISME J 3(6):685–699. https://doi.org/10.1038/ismej.2009.3

Archetti M, Ubeda F, Fudenberg D, Green J, Pierce NE, Yu DW (2011) Let the right one in: a microeconomic approach to partner choice in mutualisms. Am Nat 177(1):75–85. https://doi.org/10.1086/657622

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Barke J, Seipke RF, Gruschow S, Heavens D, Drou N, Bibb MJ, Goss RJM, Yu DW, Hutchings MI (2010) A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*. BMC Biol 8:109. https://doi.org/10.1186/1741-7007-8-109

Borewicz K, Suarez-Diez M, Hechler C, Beijers R, de Weerth C, Arts I, Penders J, Thijs C, Nauta A, Lindner C, Van Leusen E, Vaughan EE, Smidt H (2019) The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life. Sci Rep 9(1):2434. https://doi.org/10.1038/s41598-018-38268-x

Boza G, Worsley SF, Yu DW, Scheuring I (2019) Efficient assembly and long-term stability of defensive microbiomes via private resources and community bistability. PLoS Comput Biol 15(5):e1007109. https://doi.org/10.1371/journal.pcbi.1007109

Brucker RM, Bordenstein SR (2012) The roles of host evolutionary relationships (genus: *Nasonia*) and development in structuring microbial communities. Evolution 66(2):349–362. https://doi.org/10.1111/j.1558-5646.2011.01454.x

Buffie CG, Pamer EG (2013) Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 13(11):790–801. https://doi.org/10.1038/nri3535

Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM (2013) Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One 8(2):e55731. https://doi.org/10.1371/journal.pone.0055731

Chevrette MG, Carlson CM, Ortega HE, Thomas C, Ananiev GE, Barns KJ, Book AJ, Cagnazzo J, Carlos C, Flanigan W, Grubbs KJ, Horn HA, Hoffmann FM, Klassen JL, Knack JJ, Lewin GR, McDonald BR, Muller L, Melo WGP, Pinto-Tomas AA, Schmitz A, Wendt-Pienkowski E, Wildman S, Zhao M, Zhang F, Bugni TS, Andes DR, Pupo MT, Currie CR (2019) The antimicrobial potential of *Streptomyces* from insect microbiomes. Nat Commun 10(1):516. https://doi.org/10.1038/s41467-019-08438-0

Currie C, Bot A, Boomsma JJ (2003) Experimental evidence of a tripartite mutualism: bacteria protect ant fungus gardens from specialized parasites. Oikos 101(1):91–102

Currie CR (2001) A community of ants, fungi, and bacteria: a multilateral approach to studying symbiosis. Annu Rev Microbiol 55(1):357–380

Currie CR, Mueller UG, Malloch D (1999a) The agricultural pathology of ant fungus gardens. Proc Natl Acad Sci U S A 96(14):7998–8002

Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, Billen J (2006) Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. Science 311(5757):81–83. https://doi.org/10.1126/science.1119744

Currie CR, Scott JA, Summerbell RC, Malloch D (1999b) Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. Nature 398(6729):701–704. https://doi.org/10.1038/19519

Currie CR, Stuart AE (2001) Weeding and grooming of pathogens in agriculture by ants. Proc Biol Sci 268(1471):1033–1039. https://doi.org/10.1098/rspb.2001.1605

De Fine Licht HH, Boomsma JJ, Tunlid A (2014) Symbiotic adaptations in the fungal cultivar of leaf-cutting ants. Nat Commun 5:5675. https://doi.org/10.1038/ncomms6675

de Man TJ, Stajich JE, Kubicek CP, Teiling C, Chenthamara K, Atanasova L, Druzhinina IS, Levenkova N, Birnbaum SS, Barribeau SM, Bozick BA, Suen G, Currie CR, Gerardo NM (2016) Small genome of the fungus *Escovopsis weberi*, a specialized disease agent of ant agriculture. Proc Natl Acad Sci U S A 113(13):3567–3572. https://doi.org/10.1073/pnas.1518501113

Devine R, Hutchings MI, Holmes NA (2017) Future directions for the discovery of antibiotics from actinomycete bacteria. Emerg Topics Life Sci 1(1):1–12. https://doi.org/10.1042/etls20160014

Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Meta 2(2):303–336. https://doi.org/10.3390/metabo2020303

Engl T, Kroiss J, Kai M, Nechitaylo TY, Svatos A, Kaltenpoth M (2018) Evolutionary stability of antibiotic protection in a defensive symbiosis. Proc Natl Acad Sci U S A 115(9):E2020–E2029. https://doi.org/10.1073/pnas.1719797115

Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ (2018) Assembly and ecological function of the root microbiome across angiosperm plant species. Proc Natl Acad Sci U S A 115(6):E1157–E1165. https://doi.org/10.1073/pnas.1717617115

Fleming A (1929) On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of B. influenzæ. Br J Exp Pathol 10(3):226–236

Florez LV, Biedermann PH, Engl T, Kaltenpoth M (2015) Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms. Nat Prod Rep 32(7):904–936. https://doi.org/10.1039/c5np00010f

Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S (2017) The evolution of the host microbiome as an ecosystem on a leash. Nature 548(7665):43–51. https://doi.org/10.1038/nature23292

Gil-Turnes MS, Fenical W (1992) Embryos of *Homarus americanus* are protected by epibiotic bacteria. Biol Bull 182(1):105–108. https://doi.org/10.2307/1542184

Gil-Turnes MS, Hay ME, Fenical W (1989) Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science 246(4926):116–118

Gould K (2016) Antibiotics: from prehistory to the present day. J Antimicrob Chemother 71(3):572–575. https://doi.org/10.1093/jac/dkv484

Haeder S, Wirth R, Herz H, Spiteller D (2009) Candicidin-producing *Streptomyces* support leaf-cutting ants to protect their fungus garden against the pathogenic fungus *Escovopsis*. Proc Natl Acad Sci U S A 106(12):4742–4746

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321(1–2):235–257. https://doi.org/10.1007/s11104-008-9814-y

Heine D, Holmes NA, Worsley SF, Santos ACA, Innocent TM, Scherlach K, Patrick EH, Yu DW, Murrell JC, Vieria PC, Boomsma JJ, Hertweck C, Hutchings MI, Wilkinson B (2018) Chemical warfare between leafcutter ant symbionts and a co-evolved pathogen. Nat Commun 9(1):2208. https://doi.org/10.1038/s41467-018-04520-1

Hooper LV, Littman DR, Macpherson AJ (2012) Interactions between the microbiota and the immune system. Science 336(6086):1268–1273. https://doi.org/10.1126/science.1223490

Hopwood DA (2007) *Streptomyces* in nature and medicine: the antibiotic makers. Oxford University Press, New York

Kaltenpoth M (2009) Actinobacteria as mutualists: general healthcare for insects? Trends Microbiol 17(12):529–535

Kaltenpoth M, Goettler W, Dale C, Stubblefield JW, Herzner G, Roeser-Mueller K, Strohm E (2006) '*Candidatus Streptomyces philanthi*', an endosymbiotic streptomycete in the antennae of *Philanthus* digger wasps. Int J Syst Evol Microbiol 56(6):1403–1411

Kaltenpoth M, Goettler W, Koehler S, Strohm E (2010) Life cycle and population dynamics of a protective insect symbiont reveal severe bottlenecks during vertical transmission. Evol Ecol 24(2):463–477. https://doi.org/10.1007/s10682-009-9319-z

Kaltenpoth M, Göttler W, Herzner G, Strohm E (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. Curr Biol 15(5):475–479

Kost C, Lakatos T, Böttcher I, Arendholz W-R, Redenbach M, Wirth R (2007) Non-specific association between filamentous bacteria and fungus-growing ants. Naturwissenschaften 94 (10):821–828

Kroiss J, Kaltenpoth M, Schneider B, Schwinger M-G, Hertweck C, Maddula RK, Strohm E, Svatoš A (2010) Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. Nat Chem Biol 6(4):261–263

Kurth F, Mailander S, Bonn M, Feldhahn L, Herrmann S, Grosse I, Buscot F, Schrey SD, Tarkka MT (2014) *Streptomyces*-induced resistance against oak powdery mildew involves host plant responses in defense, photosynthesis, and secondary metabolism pathways. Mol Plant-Microbe Interact 27(9):891–900. https://doi.org/10.1094/MPMI-10-13-0296-R

Maldonado M, Cortadellas N, Trillas MI, Rutzler K (2005) Endosymbiotic yeast maternally transmitted in a marine sponge. Biol Bull 209(2):94–106. https://doi.org/10.2307/3593127

Marchesi JR, Ravel J (2015) The vocabulary of microbiome research: a proposal. Microbiome 3:31. https://doi.org/10.1186/s40168-015-0094-5

Marsh SE, Poulsen M, Pinto-Tomás A, Currie CR (2014) Interaction between workers during a short time window is required for bacterial symbiont transmission in *Acromyrmex* leaf-cutting ants. PLoS One 9(7):e103269

Martin-Platero AM, Valdivia E, Ruiz-Rodriguez M, Soler JJ, Martin-Vivaldi M, Maqueda M, Martinez-Bueno M (2006) Characterization of antimicrobial substances produced by *Enterococcus faecalis* MRR 10-3, isolated from the uropygial gland of the hoopoe (*Upupa epops*). Appl Environ Microbiol 72(6):4245–4249. https://doi.org/10.1128/AEM.02940-05

Martin-Vivaldi M, Pena A, Peralta-Sanchez JM, Sanchez L, Ananou S, Ruiz-Rodriguez M, Soler JJ (2010) Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. Proc Biol Sci 277(1678):123–130. https://doi.org/10.1098/rspb.2009.1377

Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, de La Cochetiere MF (2013) Development of intestinal microbiota in infants and its impact on health. Trends Microbiol 21(4):167–173. https://doi.org/10.1016/j.tim.2012.12.001

Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG (2015) The infant microbiome development: mom matters. Trends Mol Med 21(2):109–117. https://doi.org/10.1016/j.molmed.2014.12.002

Neal AL, Ahmad S, Gordon-Weeks R, Ton J (2012) Benzoxazinoids in root exudates of maize attract *Pseudomonas putida* to the rhizosphere. PLoS One 7(4):e35498. https://doi.org/10.1371/journal.pone.0035498

Newitt JT, Prudence SMM, Hutchings MI, Worsley SF (2019) Biocontrol of cereal crop diseases using streptomycetes. Pathogens 8(2):78. https://doi.org/10.3390/pathogens8020078

O'Callaghan M (2016) Microbial inoculation of seed for improved crop performance: issues and opportunities. Appl Microbiol Biotechnol 100(13):5729–5746. https://doi.org/10.1007/s00253-016-7590-9

Pathak A, Kett S, Marvasi M (2019) Resisting antimicrobial resistance: lessons from fungus farming ants. Trends Ecol Evol 34(11):974–976. https://doi.org/10.1016/j.tree.2019.08.007

Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Qin Z, Munnoch JT, Devine R, Holmes NA, Seipke RF, Wilkinson KA, Wilkinson B, Hutchings MI (2017) Formicamycins, antibacterial polyketides produced by *Streptomyces formicae* isolated from African *Tetraponera* plant-ants. Chem Sci 8:3218

Raina JB, Dinsdale EA, Willis BL, Bourne DG (2010) Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? Trends Microbiol 18(3):101–108. https://doi.org/10.1016/j.tim.2009.12.002

Raina JB, Tapiolas D, Motti CA, Foret S, Seemann T, Tebben J, Willis BL, Bourne DG (2016) Isolation of an antimicrobial compound produced by bacteria associated with reef-building corals. PeerJ 4:e2275. https://doi.org/10.7717/peerj.2275

Raina JB, Tapiolas DM, Foret S, Lutz A, Abrego D, Ceh J, Seneca FO, Clode PL, Bourne DG, Willis BL, Motti CA (2013) DMSP biosynthesis by an animal and its role in coral thermal stress response. Nature 502(7473):677–680. https://doi.org/10.1038/nature12677

Rateb ME, Ebel R (2011) Secondary metabolites of fungi from marine habitats. Nat Prod Rep 28 (2):290–344. https://doi.org/10.1039/c0np00061b

Rudrappa T, Czymmek KJ, Pare PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148(3):1547–1556. https://doi.org/10.1104/pp.108.127613

Ryan PR, Dessaux Y, Thomashow LS, Weller DM (2009) Rhizosphere engineering and management for sustainable agriculture. Plant Soil 321(1):363–383. https://doi.org/10.1007/s11104-009-0001-6

Sanders JG, Powell S, Kronauer DJ, Vasconcelos HL, Frederickson ME, Pierce NE (2014) Stability and phylogenetic correlation in gut microbiota: lessons from ants and apes. Mol Ecol 23 (6):1268–1283. https://doi.org/10.1111/mec.12611

Schatz A, Bugle E, Waksman SA (1944) Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative Bacteria. Proc Soc Exp Biol Med 55(1):66–69. https://doi.org/10.3181/00379727-55-14461

Scheuring I, Yu DW (2012) How to assemble a beneficial microbiome in three easy steps. Ecol Lett 15(11):1300–1307. https://doi.org/10.1111/j.1461-0248.2012.01853.x

Schoenian I, Spiteller M, Ghaste M, Wirth R, Herz H, Spiteller D (2011) Chemical basis of the synergism and antagonism in microbial communities in the nests of leaf-cutting ants. Proc Natl Acad Sci U S A 108(5):1955–1960

Seipke RF, Barke J, Brearley C, Hill L, Douglas WY, Goss RJ, Hutchings MI (2011) A single *Streptomyces* symbiont makes multiple antifungals to support the fungus farming ant *Acromyrmex octospinosus*. PLoS One 6(8):e22028

Seipke RF, Kaltenpoth M, Hutchings MI (2012) *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiol Rev 36(4):862–876. https://doi.org/10.1111/j.1574-6976.2011.00313.x

Selosse MA, Bessis A, Pozo MJ (2014) Microbial priming of plant and animal immunity: symbionts as developmental signals. Trends Microbiol 22(11):607–613. https://doi.org/10.1016/j.tim.2014.07.003

Sen R, Ishak HD, Estrada D, Dowd SE, Hong E, Mueller UG (2009) Generalized antifungal activity and 454-screening of *Pseudonocardia* and *Amycolatopsis* bacteria in nests of fungus-growing ants. Proc Natl Acad Sci U S A 106(42):17805. https://doi.org/10.1073/pnas.0904827106

Tamburini S, Shen N, Wu HC, Clemente JC (2016) The microbiome in early life: implications for health outcomes. Nat Med 22(7):713–722. https://doi.org/10.1038/nm.4142

van der Meij A, Worsley SF, Hutchings MI, van Wezel GP (2017) Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiol Rev 41(3):392–416. https://doi.org/10.1093/femsre/fux005

Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016) *Streptomyces* as a plant's best friend? FEMS Microbiol Ecol 92(8):fiw119

Wang L, Feng Y, Tian J, Xiang M, Sun J, Ding J, Yin WB, Stadler M, Che Y, Liu X (2015) Farming of a defensive fungal mutualist by an attelabid weevil. ISME J 9(8):1793–1801. https://doi.org/10.1038/ismej.2014.263

Worsley SF, Innocent TM, Heine D, Murrell JC, Yu DW, Wilkinson B, Hutchings MI, Boomsma JJ, Holmes NA (2018) Symbiotic partnerships and their chemical interactions in the leafcutter ants (Hymenoptera: Formicidae). Myrmecological News 27:59–74

Zhang Y, Ruyter-Spira C, Bouwmeester HJ (2015) Engineering the plant rhizosphere. Curr Opin Biotechnol 32:136–142. https://doi.org/10.1016/j.copbio.2014.12.006

Zivkovic AM, German JB, Lebrilla CB, Mills DA (2011) Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci U S A 108(Suppl 1):4653–4658. https://doi.org/10.1073/pnas.1000083107