

REVIEW ARTICLE

***Streptomyces* as symbionts: an emerging and widespread theme?**

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Abstract

Streptomyces bacteria are ubiquitous in soil, conferring the characteristic earthy smell, and they have an important ecological role in the turnover of organic material. More recently, a new picture has begun to emerge in which streptomycetes are not in all cases simply free-living soil bacteria but have also evolved to live in symbiosis with plants, fungi and animals. Furthermore, much of the chemical diversity of secondary metabolites produced by *Streptomyces* species has most likely evolved as a direct result of their interactions with other organisms. Here we review what is currently known about the role of streptomycetes as symbionts with fungi, plants and animals. These interactions can be parasitic, as is the case for scab-causing streptomycetes, which infect plants, and the *Streptomyces* species *Streptomyces somaliensis* and *Streptomyces sudanensis* that infect humans. However, in most cases they are beneficial and growth promoting, as is the case with many insects, plants and marine animals that use streptomycete-produced antibiotics to protect themselves against infection. This is an exciting and newly emerging field of research that will become increasingly important as the search for new antibiotics switches to unusual and under-explored environments.

Introduction

Around 130 years ago, the German naturalist Heinrich Anton de Bary used the term 'symbiosis' to describe any stable association between different organisms over a significant portion of their lives (de Bary, 1879). In the broadest sense, this definition includes the whole continuum ranging from mutually beneficial interactions via commensalisms to parasitic relationships (Douglas, 1994). While parasitisms have traditionally been studied intensively because of their direct medical implications, mutualisms have only in the past decades begun to attract considerable attention because of their enormous ecological and evolutionary importance (Moran, 2006). As a result of intensive research efforts, it is becoming increasingly apparent that the vast majority of multicellular animals as well as many plants and fungi engage in mutualistic interactions with microorganisms that are often essential for successful growth and reproduction of the host (Margulis & Fester, 1991; Moran, 2006). Because

of their abundance in terrestrial and marine habitats and their metabolic versatility, bacteria are the most common symbiotic partners of eukaryotes.

The phylum Actinobacteria is one of the largest taxonomic units among the 18 major lineages of bacteria, and its divergence from other bacterial phyla is so ancient that it is currently not possible to identify their most closely related group (Ventura *et al.*, 2007). Actinobacteria are enormously important to human medicine, agriculture and food production, and key to this is their ability to interact with other organisms, an area which has largely been overlooked in the last 150 years of their study. In humans, actinobacteria are estimated to make up around one-third of the gut microbiota, most notably *Bifidobacterium* species, which are beneficial to human health and nutrition (Miao & Davies, 2010). Conversely, *Mycobacterium tuberculosis*, carried by two billion people in the world, is the causative agent of tuberculosis, one of the oldest infectious diseases of humans that is estimated to kill several million people every year.

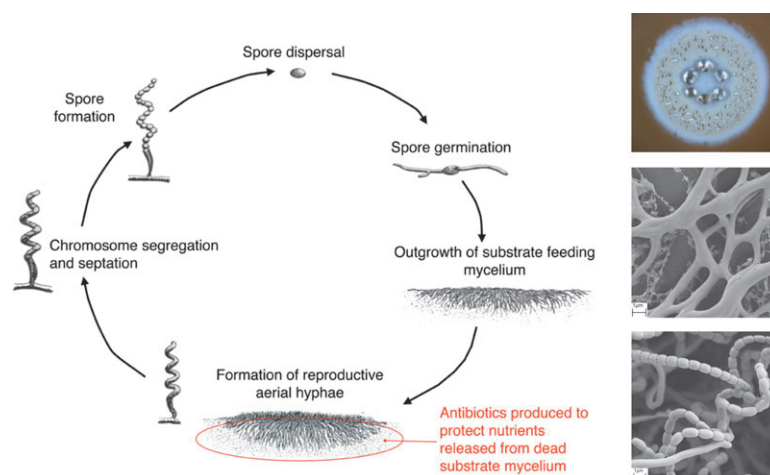


Fig. 1. The *Streptomyces* life cycle begins with spore germination and outgrowth of a feeding substrate mycelium. In response to nutritional and other stress signals, *Streptomyces* spp. produce reproductive aerial hyphae, which undergo cell division to form spores. Antibiotic production is usually linked to differentiation, as shown, although not all *Streptomyces* antibiotics are produced in the stationary phase. The pictures on the right show a colony of *Streptomyces* spp. isolated from *Allomerus* ants at 40× magnification (top), and scanning electron micrographs of substrate mycelium (middle) and aerial hyphae and spore chains (bottom) of *Streptomyces coelicolor* grown on soya flour mannitol agar, both at 400× magnification.

Arguably, the best studied genus of actinobacteria is *Streptomyces*, which have complex developmental life cycles (Flårdh & Buttner, 2009) and produce numerous secondary metabolites that are used in human medicine as anti-infectives, antitumour and immunosuppressant drugs (Challis & Hopwood, 2003) (Fig. 1). The bioactive compounds made by these bacteria, and by filamentous fungi, revolutionized medicine in the 20th century not just by curing previously fatal diseases, but also by enabling potentially life-saving procedures such as heart surgery and transplantation to be performed. Unfortunately, efforts to discover and exploit natural products have been in decline while drug-resistant pathogens are emerging and we are faced with the prospect of having no effective antibiotics to treat infectious diseases, less than 100 years after the first antibiotic was discovered (Cooper & Shlaes, 2011).

The discovery of *Streptomyces* spp. natural products was pioneered by Selman Waksman, a soil microbiologist who, after two decades of studying the actinomycetes as members of the soil microbial community, was inspired by the exploitation of Alexander Fleming's discovery of the first natural product antibiotic, penicillin. Waksman and his graduate student Albert Schatz went on to discover streptomycin, a product of *Streptomyces griseus* that was the first effective antibiotic against *M. tuberculosis* (Schatz & Waksman, 1944). The *Streptomyces*-produced secondary metabolite called geosmin (literally 'earth smell') does not have antibiotic activity, but gives the soil its characteristic smell, and provides an indication of just how widespread these bacteria are in the soil. The adap-

tive significance of geosmin is not known, but its production is a well-conserved trait in *Streptomyces* spp. so presumably it has an important role (Hopwood, 2007). The gene underlying geosmin production is well conserved among *Streptomyces* species (Gust *et al.*, 2003).

Streptomyces bacteria are well adapted to life in the soil where they grow as a substrate mycelium made of multiple hyphae that grow by tip extension and branch through the soil searching for nutrients. They secrete large numbers of enzymes that break down insoluble organic polymers, including chitin and cellulose, into substituent sugars for binding and uptake by multiple ABC transporters (Bertram *et al.*, 2004; Chater *et al.*, 2010; Thompson *et al.*, 2010). The diversity of secondary metabolites made by *Streptomyces* species has led some scientists to argue they are not simply chemical weapons used to kill other soil organisms but are signalling molecules which, at low doses, can modulate the transcription and translation of genes in target organisms (Yim *et al.*, 2007; Davies & Davies, 2010). Although the concentrations of secondary metabolites in the natural environment are rarely known, it seems likely that antibiotic compounds are usually present in subinhibitory concentrations (Davies *et al.*, 2006; Martínez, 2008; Aminov, 2009; Baquero & Ortega, 2009). Whatever the natural function of these secondary metabolites, it appears that their diversity evolved through interactions among bacteria and other organisms, and humans have barely begun to sample or understand this chemical diversity (Clardy *et al.*, 2009).

Streptomyces bacteria carry resistance to their own antibiotics to avoid suicide and, under selective pressure

(i.e. persistent exposure to antibiotics), these resistance genes can spread to other soil bacteria and to pathogenic bacteria via horizontal gene transfer (e.g. Cundliffe, 1989; Aminov & Mackie, 2007). For example, there is good evidence that the resistance genes for the glycopeptide antibiotic vancomycin found in vancomycin-resistant enterococci were acquired over a period of three decades or more from the glycopeptide-producing strains found in the soil and then took another decade or more to transfer to methicillin-resistant *Staphylococcus aureus* (Weigel *et al.*, 2003; Hong *et al.*, 2008). More recently it has been proposed that the soil offers a reservoir of antibiotic resistance genes, called the soil resistome, that makes the evolution of drug resistance in pathogenic bacteria inevitable (Wright, 2010). *Streptomyces* bacteria are also resistant to nitric oxide (NO), a natural antimicrobial gas that is produced by the human immune system to kill invading microorganisms and by the nitrate reductases and NO synthases of soil microorganisms (Tucker *et al.*, 2010). It has been hypothesized that the NO resistance genes found in pathogenic bacteria and human commensals also originated in soil bacteria like *Streptomyces* (Tucker *et al.*, 2008, 2010). Many Gram-positive soil bacteria encode NO synthases, including plant pathogenic *Streptomyces* spp., and NO has been shown to confer antibiotic resistance, suggesting some soil bacteria produce NO to combat the natural product antibiotics made by other soil microorganisms (Gusarov *et al.*, 2009). Interestingly, NO resistance has also been implicated in the establishment of a stable symbiosis between *Vibrio fischeri* and the bobtail squid, *Sepiolo atlantica* (Davidson *et al.*, 2004; Dunn *et al.*, 2010; Wang *et al.*, 2010; Wang & Ruby, 2011).

There is increasing evidence that streptomycetes are not just free-living soil bacteria, but also form symbioses with other organisms, most notably plants and invertebrates. With hindsight, it seems obvious that *Streptomyces* spp., which are ubiquitous in soils, sediments and seawater, would have evolved to interact, beneficially or otherwise, with the roots of plants and with multicellular soil and marine dwelling animals. In several cases, *Streptomyces* spp. form protective mutualistic symbioses in which the host feeds and protects the bacteria and in return the bacteria provide antibiotics to protect the host, or the host resources, from pathogens (Kaltenpoth, 2009). Other genera of actinobacteria that are known to form mutualistic symbioses with higher organisms include *Frankia* and *Micromonospora*, which form nitrogen fixing actinonodules in trees and shrubs (Kucho *et al.*, 2010; Trujillo *et al.*, 2010). In addition, antibiotic-producing *Pseudonocardia*, *Amycolatopsis* and *Saccharopolyspora* species engage in protective mutualisms with insects (Kaltenpoth, 2009). *Streptomyces* spp. can also cause opportunistic infections known as actinomycetomas in humans (Ahmed *et al.*,

2007), and a few species are dedicated pathogens of plants (Loria *et al.*, 2006). If the diversity of secondary metabolites produced by streptomycetes and other microorganisms did evolve as a result of interactions with other organisms, including symbioses, this provides a good reason to promote research in this emerging area. The symbioses formed between *Streptomyces* spp. and fungi, plants and animals will be the subject of this review.

Streptomycetes and their interactions with invertebrates

In recent years, it has become clear that streptomycetes and other actinobacteria are well adapted to living in symbiosis with invertebrates where they mostly play a protective role, producing antibiotics that are used to defend the host's larvae or food source against infections by pathogens (Kaltenpoth, 2009). Although specific examples of insects associated with actinobacteria are relatively rare, it has been proposed that these interactions are widespread in the insect world, and the number of documented examples is likely to increase dramatically over the next few years (Kaltenpoth, 2009). In addition to forming symbioses with insects, actinobacteria also form stable associations with marine invertebrates, and there are many reports of interactions between *Streptomyces* species and marine sponges and cone snails. This area of research has largely been stimulated by the search for new classes of natural product antibiotics and anticancer agents in the marine environment (Piel, 2004, 2009; Gulner & Moore, 2009) (Fig. 2).

Marine invertebrates and streptomycetes

Marine sponges are among the oldest multicellular animals and are emerging as the major marine source of novel secondary metabolites, at least some of which are made by bacteria. The combined mass of bacteria associated with the animals can constitute up to 40% of the total sponge biomass and is two or three orders of magnitude higher than the mass of bacteria in seawater. Although some of these interactions are transient, there is also strong evidence for stable interactions between bacteria and sponges, both mutualistic and parasitic (Taylor *et al.*, 2007a). Remarkably, it has been shown that distantly related sponges from diverse geographical locations share a large proportion of their microbiota (Hentschel *et al.*, 2002). Bacteria variously serve as a food source for marine sponges or as a disease-causing agent, but actinobacteria and other antibiotic-producing bacteria associated with marine sponges also offer protection against disease (Selvin *et al.*, 2009). In terms of their microbiota, marine sponges are divided into high microbial

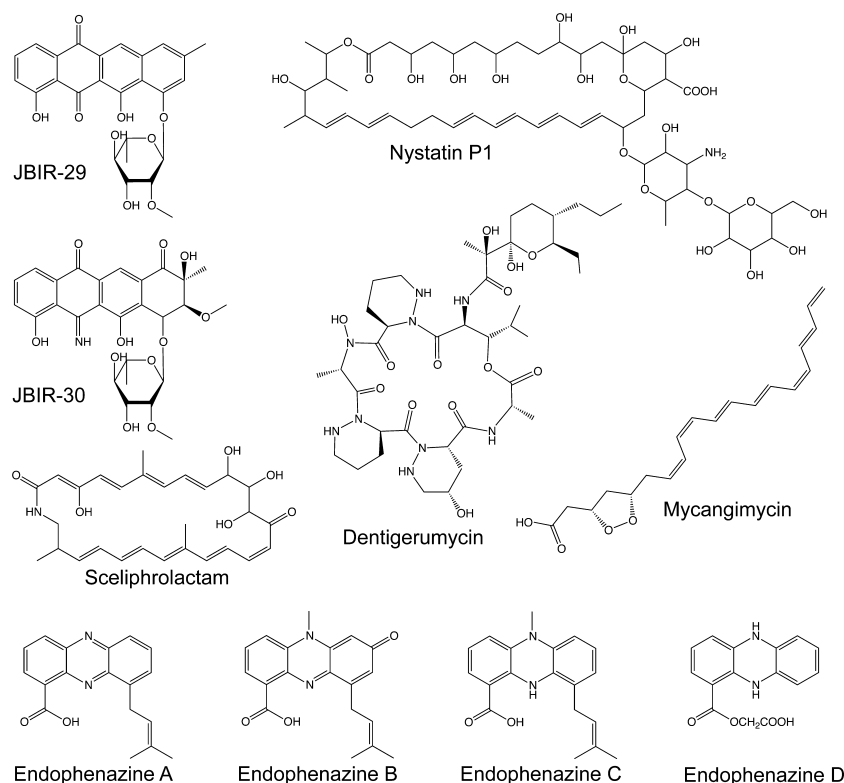


Fig. 2. Structures, or predicted structures, of novel antibiotics isolated from *Streptomyces* spp. and closely related actinobacteria living in symbiosis with insects and marine invertebrates. Stereochemistry is shown for compounds where it is known. JBIR29 and JBIR30 were isolated from *Streptomyces* spp. associated with a marine sponge (Khan *et al.*, 2011). Nystatin P1 was isolated from a *Pseudonocardia* species associated with the attine ant *Acromyrmex octospinosus*. The mycosamine sugar has an additional hexose sugar attached, as judged by LC-MS/MS, and the most likely structure is shown, although this has not been confirmed experimentally (Barke *et al.*, 2010). Dentigerumycin was isolated from a *Pseudonocardia* species associated with the attine ant *Apterostigma dentigerum* (Oh *et al.*, 2009a); mycangimycin was isolated from a *Streptomyces* species associated with the fungus-growing pine beetle *Dendroctonus frontalis* (Oh *et al.*, 2009b); sceliphrolactam was isolated from *Streptomyces* species associated with mud dauber wasps (Oh *et al.*, 2011; Poulsen *et al.*, 2011) and the endophenazine compounds A–D were isolated from *Streptomyces* endosymbiont species taken from four different arthropods (Gebhardt *et al.*, 2002).

abundance and low microbial abundance sponges (Vaculet & Donadey, 1977) and both groups are associated with actinobacteria, including multiple *Streptomyces* spp. (Kamke *et al.*, 2010). A recent study of the marine sponge *Haliclona* spp. (Fig. 2) isolated from the coastal waters of Japan yielded 162 strains of actinobacteria of which 131 belonged to the genus *Streptomyces* (Khan *et al.*, 2011). Of these 131, six phylogenetically new strains were screened for the production of secondary metabolites and several unique nonribosomal peptide synthetase-generated antibiotics were identified along with novel analogues belonging to known classes of antibiotics, one of which exhibited strong activity against anticancer-drug-resistant cell lines (Khan *et al.*, 2011) (Fig. 2). It is worth noting that this study set out to isolate *Streptomyces* spp., which perhaps explains their over-representation, although the authors did report isolation of six other genera of actinobacteria in their study. Another study of actinobacteria

associated with the marine sponge *Haliclona*, in this case isolated from the shallow waters of the South China Sea, also found that *Streptomyces* species are dominant using culture-dependent approaches and represented 60% of the actinobacteria isolated in that study (Jiang *et al.*, 2007). In contrast, more general studies of the bacterial communities associated with *Haliclona* using 16S rDNA libraries failed to identify many actinobacterial species, perhaps because of the methodology employed (Jiang *et al.*, 2007). In similar studies on the actinobacteria associated with the marine sponge *Hymeniacidon perleve*, also isolated from the South China Sea, 24 species were cultured, identified and assigned to six genera, one of which was *Streptomyces*. In fact *Streptomyces* was one of only two genera identified in this sponge using both culture-dependent and culture-independent techniques (Sun *et al.*, 2010). Given the estimated 1400 species of marine sponge, much wider sampling will be required to

investigate the true diversity of *Streptomyces* spp. associated with these ancient animals. However, it is important to note that *Streptomyces* spp. have been identified in all of the sponges that have been analysed specifically for the presence of actinobacteria, and there is good reason to speculate that their association with marine sponges is widespread and protective. In the most comprehensive survey of the microbial life associated with marine sponges carried out to date, Taylor *et al.* (2007b) found that actinobacteria are associated with at least 28 recognized genera of marine sponges and other studies have shown that the dominant actinobacteria associated with sponges belong to the genera *Streptomyces* and *Mycobacterium*. The idea that *Streptomyces* spp. are stably associated with marine sponges is consistent with the emerging evidence that shows *Streptomyces* spp. to be *bona fide* marine actinomycetes and not simply a result of terrestrial run-off into the sea (Moran *et al.*, 1995; Bull *et al.*, 2005).

Recently, marine cone snails have also been discovered to harbour a diverse community of actinobacteria, among them many species of *Streptomyces* (Peraud *et al.*, 2009). Several of the isolated actinomycete strains produced compounds with neurological and/or antifungal bioactivity (Peraud *et al.*, 2009; Lin *et al.*, 2010), but their functions *in vivo* are still unknown. Unfortunately, all of the studies on actinobacteria associated with marine invertebrates have been driven by the need to find new antibiotics, and the biological role of *Streptomyces* spp. in this environment has not been addressed experimentally. It is tempting to speculate that *Streptomyces* spp. live in mutualistic associations with marine animals and protect them against bacterial infection. However, despite evidence that the microbial populations of sponges are stable and highly conserved, very little work has been performed to investigate symbioses between individual species and to elucidate the function of the symbionts in the live sponge (Webster & Blackall, 2008).

Solitary wasps and their *Streptomyces* symbionts

Beewolf solitary digger wasps (*Philanthus* spp.) form a mutually beneficial endosymbiosis with a *Streptomyces* strain named '*Candidatus Streptomyces philanthi*' (Kaltenpoth, 2006). Female beewolves nest by digging underground burrows in the soil, and they hunt honeybees as a source of food for their larvae (Fig. 3). They paralyse their honeybee prey and place between one and five of these paralysed honeybees in their brood cells for the larvae to feed upon. Once the larvae are sated, they spin cocoons in which they spend the winter before emerging in early summer. However, because the conditions in the



Fig. 3. Left, a female beewolf digger wasp (*Philanthus coronatus*) with a paralysed prey bee. Right, '*Candidatus Streptomyces philanthi*' from the antennal gland secretion of *Philanthus triangulum*, stained with the Cy3-labelled probe SPT177 (Kaltenpoth *et al.*, 2005).

brood cells are warm and humid, there is a constant threat of bacterial or fungal infection from the surrounding soil, which could destroy the larvae. As an adaptation that prevents infection by bacteria and fungi, female beewolves have evolved a unique and remarkable mutualistic symbiosis with *Streptomyces* bacteria that grow inside the specialized antennal glands of the female wasps (Kaltenpoth *et al.*, 2005) (Fig. 3). Once the female wasp has excavated her burrow, she secretes large amounts of a white substance out of her antennal glands, which is largely made up of these *Streptomyces* endosymbionts (Kaltenpoth *et al.*, 2005; Kroiss *et al.*, 2010). The larvae take up the symbiotic *Streptomyces* bacteria from the mother's secretion droplets in the brood cell and incorporate them into the cocoons they are spinning. A recent work extracted cocoons from a laboratory population of digger wasps and identified nine antibiotics using LC-MS and NMR. Furthermore, high and evenly spread concentrations of the three most abundant antibiotics on the outside of the cocoons were directly visualized using laser desorption/ionization mass spectrometric imaging, thus offering direct evidence that these antibiotics are used by the larvae as protection against invading microbial pathogens *in situ*. Finally, the cocktail of these antibiotics had strong inhibitory activity against all the pathogenic microorganisms that were tested in agar-diffusion bioassays (Kroiss *et al.*, 2010). This use of multidrug therapy by beewolf digger wasps probably has the combined advantages of offering greater protection against a wide range of potential pathogens and also reducing the evolution of drug resistance in pathogens that frequently invade the brood cells.

Following the discovery of symbiotic *Streptomyces* spp. associated with *Philanthus* digger wasps, the solitary mud dauber wasps *Sceliphron caementarium* and *Chalybion californicum* were also shown to be associated with

Streptomyces spp. *Sceliphron caementarium* construct nests using mud they collect from water puddles, and they partition these nests into multiple cells, each containing a single egg and a paralysed prey that serves as food for their developing larvae. This is reminiscent of the digger wasps except the dauber wasp prey is typically a paralysed spider rather than a honeybee. In contrast to this, *C. californicum* dauber wasps re-utilize established dauber nests, discarding the contents of the cells and replacing them with their own eggs and paralysed prey (Poulsen *et al.*, 2011). In a study of the bioactive compounds produced by the wasp-associated *Streptomyces* isolates, the authors identified 11 structurally diverse secondary metabolites, including one polyoxygenated macrocyclic lactam compound that is novel (Poulsen *et al.*, 2011) (Fig. 2). This offers evidence that exploring the unusual niche of insect–actinobacterial symbioses has the potential to yield novel antibiotics (Clardy *et al.*, 2009; Kaltenpoth, 2009).

Attine (fungus-growing) ants and their defensive actinobacteria

Fungiculture has evolved several times in the insect world and has been best explored in the higher attine ants, which are endemic to South and Central America and to the southern United States. The ancestor of these ants evolved the ability to cultivate fungus as a food source around 50 million years ago, leading to the monophyletic tribe Attini, which comprises 12 genera with more than 230 species. The genera *Acromyrmex* and *Atta* evolved 8–12 million years ago and form a branch of the higher attines known as leaf-cutter ants, which are characterized by large colonies of up to several million individuals (Schultz & Brady, 2008). The well-studied species *Acromyrmex octospinosus* forms a mutualism with a single basidiomycete fungus, *Leucoagaricus gongylophorus*, in which they exchange food as well as protection and transport services (Fig. 4). The fungal garden can be parasitized by a number of fungal pathogens, most notably those of the genus *Escovopsis*, and there is evidence that the fungal cultivar produces antibiotics to defend itself (Wang *et al.*, 2002). The ant workers also protect their fungal gardens through a combination of grooming and weeding (Currie & Stuart, 2001), production of their own antimicrobials through metapleural gland secretions (Fernández-Marín *et al.*, 2006; Yek & Mueller, 2011) and the application of antimicrobials produced by symbiotic actinobacteria (Currie *et al.*, 1999; Haeder *et al.*, 2009; Oh *et al.*, 2009a; Sen *et al.*, 2009; Barke *et al.*, 2010).

The long-standing model suggests that bacteria from the genus *Pseudonocardia* co-evolved with the ants and are transmitted vertically by the queens along with the fungal cultivar (Currie *et al.*, 1999). Phylogenetic studies



Fig. 4. *Acromyrmex octospinosus* leaf-cutter ants tending their fungus garden. These ants grow the fungus *Leucoagaricus gongylophorus* as the sole food source for their colony and use antibiotics made by symbiotic actinobacteria to suppress the growth of other microorganisms.

not only found evidence for a certain degree of specificity in the ant–*Pseudonocardia* association, but also revealed repeated uptake and/or horizontal transmission events of the symbiotic bacteria (Mueller *et al.*, 2008, 2010; Cafaro, 2011). Some support for the hypothesis of co-evolution between ants and *Pseudonocardia* was provided by the observation that the bacteria are cultivated in gland-associated cuticular crypts, and that the structure and complexity of these crypts have changed during the evolutionary history of the symbiosis (Currie *et al.*, 2006). However, recently evidence has accumulated that suggests attine ants are associated with a more complex community of actinobacteria, including members of the genera *Streptomyces* and *Amycolatopsis*, and that antibiotic-producing actinomycetes can be horizontally acquired through male dispersal and sampling of actinomycetes from the soil (Kost *et al.*, 2007; Sen *et al.*, 2009; Barke *et al.*, 2010, 2011). Candidicin- and antimycin-producing *Streptomyces* spp. have been shown to be common mutualists of *Acromyrmex* ants and were probably acquired relatively recently from the environment (Haeder *et al.*, 2009; Barke *et al.*, 2010; Seipke *et al.*, 2011a).

The identities of the antibiotics produced by ant-associated actinobacteria remain largely unknown for most attine species. However, several antifungal and antibacterial compounds have been identified in *Apterostigma* and *Acromyrmex*: a previously unknown antifungal named dentigerumycin that is produced by a *Pseudonocardia* species isolated from the lower attines *Apterostigma dentigerum* (Oh *et al.*, 2009a); a novel variant of nystatin named nystatin P1 that is produced by *Pseudonocardia* species isolated from *A. octospinosus* (Barke *et al.*, 2010).

(Fig. 2); candicidin, a well-known antifungal compound that is produced by *Streptomyces* species isolated from ants belonging to the genus *Acromyrmex* (Haeder *et al.*, 2009; Barke *et al.*, 2010; Seipke *et al.*, 2011a); and antimycins, another group of well-known antifungal compounds that inhibit the electron transport chain (Schoenian *et al.*, 2011; Seipke *et al.*, 2011a). In a big step forwards, the *Streptomyces*-produced antibacterial valinomycin was also recently identified on the surface of attine worker ants, providing the first evidence that these bacteria are producing antibiotics while living on the ants. The same authors also identified actinomycins being produced by these *Streptomyces* strains (Schoenian *et al.*, 2011). It has been suggested previously that the leaf-cutting ant mutualism could be a source of novel antimicrobials, but most of the antibiotics discovered to date in this system are either well known or belong to known classes. This field appears to be challenged with the same rediscovery problem that has dogged other efforts to find new antibiotics. However, two recent studies revealed that *Streptomyces* symbionts isolated from *A. octospinosus* and *Acromyrmex volcanus* make multiple antifungal compounds, including antimycins and candicidin (Schoenian *et al.*, 2011; Seipke *et al.*, 2011a). Disruption of the biosynthetic gene clusters for antimycin and candicidin in one of these symbionts did not abolish antifungal activity against the nest pathogen *Escovopsis*, and analysis of the genome sequence did not reveal any other known antifungal biosynthetic pathways. This suggests that the compound(s) and/or the genes encoding the biosynthesis of these compound(s) are novel to science and certainly to the attine ant system (Seipke *et al.*, 2011a, b).

Southern pine beetles and streptomycetes

Bark beetles are serious forestry pests that reproduce in the bark and phloem and cause considerable damage to their host trees. In a system that is similar to the attine ants, the southern pine beetle *Dendroctonus frontalis* lives in a mutual symbiosis with the fungus *Entomocorticium*, which it relies on for food. The adult beetles carry this fungus in a specialized compartment called a mycangium, which they use to inoculate galleries carved into the bark of their host pine trees. As with the attine ant mutualism, the fungus is susceptible to infection by an antagonistic fungus, in this case *Ophiostoma minus*, which can out-compete the cultivar fungus and thereby severely disrupt the development of the beetle larvae (Hofstetter *et al.*, 2006). A recent study reported that this mutualism is protected by *Streptomyces* bacteria that are closely related to *Streptomyces thermosacchari*. *Streptomyces* bacteria that were isolated from the beetles and their mycangia had strong activity against the pathogenic fungus *O. minus*

and weak activity against the mutualist fungus *Entomocorticium* (Scott *et al.*, 2008). Mass spectrometric analysis of the compounds made by this mutualist *Streptomyces* species identified a previously unknown polyene antifungal that the authors named mycangimycin (Scott *et al.*, 2008) (Fig. 2). Undoubtedly, the same species produces multiple other antibiotics, analogous with all known *Streptomyces* spp. Subsequent work revealed that three strains of *Streptomyces* can be consistently isolated from multiple *Dendroctonus* species and their galleries collected from separate locations throughout the North American range of the genus, suggesting that this is a widespread association between bark beetles and antifungal-producing *Streptomyces* species (Hulcr *et al.*, 2011). This work offers additional evidence that actinobacteria are widely used by fungus-farming insects for the protection of their resources (Kaltenpoth, 2009).

Allomerus ants and their actinobacteria

Allomerus spp., like the tribe Attini, are in the ant subfamily Myrmicinae, but they differ from the attines because they are specialist plant symbionts that employ a unique mechanism of ambush hunting (Dejean *et al.*, 2005). *Allomerus* ants use a sooty mould fungus of the order *Chaetothyriales* to build a galleried structure along the stems and branches of their host plants and then they hide in the galleries and await their prey (Dejean *et al.*, 2005; Ruiz-González *et al.*, 2010) (Fig. 5). Although vertical transmission of the fungus from queen to queen has not been verified experimentally, it seems likely that this specific interaction between a fungus and ants of the genus *Allomerus* is the result of fungiculture, which is thought to be widespread in ant-plant systems (Defosse *et al.*, 2009). The only fungus that has been observed growing in the traps of *Allomerus* ant-plants are those belonging to the symbiotic sooty mould fungus, but spores from 44 other fungi have been isolated from trap material, suggesting that the ants employ mechanisms to



Fig. 5. *Allomerus* ants in one of the galleries they construct on the undersides of branches on their host ant-plants *Hirtella* and *Cordia*. The ants use plant trichomes, which can be seen growing perpendicular to the top of the branch, to construct the galleries, which are then strengthened by the growth of a sooty mould of the order *Chaetothyriales*.

suppress the growth of alternative, and potentially parasitic, fungi (Ruiz-González *et al.*, 2010). Spores from non-cultivar fungi have also been found in the infrabuccal pellets of *Allomerus* workers, suggesting that, like the leaf-cutter ants, *Allomerus* ants are actively removing fungal contaminants through grooming and weeding (Ruiz-González *et al.*, 2010). Significantly, it was recently demonstrated that *Allomerus* ants are also associated with antifungal-producing actinobacteria from the genera *Amycolatopsis* and *Streptomyces* (Seipke *et al.*, 2011c). Although it has not been proved that these actinobacteria are mutualists of *Allomerus* ants, it seems likely that the system is similar to that of the attines in that the *Allomerus* worker ants use the antifungal compounds produced by *Streptomyces* and *Amycolatopsis* strains as weedkillers to suppress fungal pathogens in their ant-plant traps (Seipke *et al.*, 2011c). Researchers are at the early stages of elucidating the relationships between the plant-ants, their cultivar fungus and actinobacteria, but this is the first experimental evidence that a system of fungus-growing ants other than the attines may use actinobacteria in a protective role.

Streptomyces and other terrestrial invertebrates

Members of the genus *Streptomyces* have been identified in the guts of various arthropod species, including termites, beetles, millipedes, wood lice and earthworms (Pasti & Belli, 1985; Pasti *et al.*, 1990; Bignell & Anderson, 1991; Schäfer *et al.*, 1996; Krastel *et al.*, 2002; Watanabe *et al.*, 2003; Jayasinghe & Parkinson, 2009; Zhou *et al.*, 2011). Although *Streptomyces annulatus* was repeatedly isolated from different arthropod hosts and found to produce endophenazines with antibacterial and antifungal activity *in vitro* (Gebhardt *et al.*, 2002; Krastel *et al.*, 2002) (Fig. 2), to date nothing is known about the intimacy of the symbiotic relationship with the host or the functional role of the bacteria *in vivo*. Similarly, *Streptomyces* spp. have been isolated from termite guts and suggested to play a role in cellulose or lignin degradation, but their functional significance within the host remains enigmatic (Pasti & Belli, 1985; Pasti *et al.*, 1990; Bignell & Anderson, 1991; Schäfer *et al.*, 1996). Interestingly, Kristufek *et al.* (2001) observed enhanced proliferation of the fungus-feeding oligochaete *Enchytraeus crypticus* when it was artificially infected with *Streptomyces lividans*, which was attributed to the degradation of the fungal cell walls by bacterial chitinases (Bühmann *et al.*, 1999). Thus, *Streptomyces* spp. may be widespread inhabitants of invertebrates' guts and possibly contribute to the degradation of polymeric carbohydrates or to antimicrobial defence (Fig. 7).

Interactions between streptomycetes and vertebrates

To our knowledge, there are no reports of stable beneficial interactions between *Streptomyces* bacteria and vertebrates. However, it is worth noting here that two species of *Streptomyces* are well documented as causes of actinomycetoma in humans (Quintana *et al.*, 2008). These species, *Streptomyces sudanensis* and *Streptomyces somaliensis*, and the disease they cause are largely under-studied and little known outside the countries in which they occur, despite the severity of the outcome, which includes limb deformities that can lead to poor social and economical prospects (Ahmed *et al.*, 2007). Actinomycetoma is restricted to the tropics where infections arise through mild trauma, usually caused by thorns or similar structures on plants carrying the bacteria. This is followed by growth of the filamentous *Streptomyces* bacteria into the skin, then the subcutaneous tissue and eventually into the bone where most of the damage occurs. However, the soft tissue infection often leads to secondary infections by staphylococci and streptococci, and in immunocompromised individuals can lead to bacteremia in which the *Streptomyces* bacteria spread to the bloodstream (Joseph *et al.*, 2010).

Streptomyces as plant symbionts

Streptomyces spp. display lifestyles ranging from benign saprophytes to beneficial plant endosymbionts to plant pathogens. They have a competitive advantage over many other microorganisms in soil ecosystems, because of their filamentous and sporulating lifestyle, which allows them to persist during harsh environmental conditions. The filamentous lifestyle of streptomycetes also affords bacteria of this genus the ability to colonize nearby roots and subsequently directly penetrate plant cells to gain entry into the host, leading to endophytic and pathogenic phenotypes (Coombs & Franco, 2003a, b; Franco *et al.*, 2007; Joshi *et al.*, 2007a).

Plant pathogenic streptomycetes

Phytopathogenic *Streptomyces* spp. are a rarity in the genus *Streptomyces* and comprise only a handful of species, including the well-studied examples *Streptomyces turgidiscabies*, *Streptomyces acidiscabies*, *Streptomyces scabies* and *Streptomyces ipomoeae*. These pathogens have a broad host range and can infect plants such as tomato (Fig. 6), but are mostly known for their ability to cause necrotic scab-like lesions on economically important root and tuber crops such as potato (Loria *et al.*, 2003). Scab-causing streptomycetes colonize root structures and subsequently



Fig. 6. Tomato plants mock infected (left) or infected with *Streptomyces scabies* (right). Infection by *S. scabies* greatly reduces the complexity of the root system and stunts plant growth.

directly penetrate plant cells and grow both inter- and intracellularly within the plant host (Loria *et al.*, 2003; Joshi *et al.*, 2007a). The success of these pathogens is linked to the ability to manipulate the plant host by producing a suite of phytotoxins, secreted proteins and phytohormones that manipulate host physiology to their advantage.

Pathogenicity appears to be an acquired phenotype, as several virulence factors reside on a mobilizable pathogenicity island that, when transferred to a saprophytic streptomycete, confers the ability to cause disease (Kers *et al.*, 2005). Pathogenicity requires the production of the nitrated phytotoxin thaxtomin, a dipeptide that is a potent inhibitor of cellulose biosynthesis and triggers the release of cello-oligosaccharides from expanding plant tissue (Scheible *et al.*, 2003; Johnson *et al.*, 2007). The biosynthesis of thaxtomin is well characterized (Healy *et al.*, 2000, 2002; Kers *et al.*, 2004; Johnson *et al.*, 2009; King & Calhoun, 2009), and thaxtomin production is controlled by TxtR, an AraC/XylS-family regulator that binds cellobiose (Joshi *et al.*, 2007b). Development of full disease symptoms requires Nec1, a unique necrogenic protein with no known homologue, which is required for colonization of plant roots and has been proposed to be an inhibitor of the host defence response (Joshi *et al.*, 2007a).

Recently, genome sequencing has extended our understanding of virulence mechanisms of phytopathogenic *Streptomyces* spp. beyond that of thaxtomin and Nec1 to define the pathogenome, which includes secreted proteins and secondary metabolites that are predicted to contribute to plant-microbe interactions (Bignell *et al.*, 2010b; Huguet-Tapia *et al.*, 2011). Scab-causing streptomycetes

possess a conserved saponin-degrading enzyme that has been characterized in *S. scabies* 87-22 (Kers *et al.*, 2005; Seipke & Loria, 2008). *S. turgidiscabies* Car8 possesses a biosynthetic gene cluster for cytokinin, a phytohormone that the pathogen produces that causes the formation of leafy galls (Joshi & Loria, 2007). Multiple virulence determinants are secreted by *S. scabies* 87-22 via the twin-arginine transport (Tat) pathway and are required for full development of disease symptoms (Joshi *et al.*, 2010). These Tat-dependent virulence determinants include a lipoprotein, which is essentially a cell surface protein tethered to the outer surface of the membrane in Gram-positive bacteria via a lipid modification (Hutchings *et al.*, 2009). Subsequent disruption of lipoprotein biogenesis in *S. scabies* revealed a moderate effect on virulence in strains that cannot process and display lipoproteins correctly (Widdick *et al.*, 2011). Genome mining of *S. scabies* 87-22 for the production of secondary metabolites resulted in the identification of a biosynthetic pathway that produces sterol-like molecules called hopanoids (Seipke & Loria, 2009), a pyochelin siderophore (Seipke *et al.*, 2011d) and a coronafacic acid-like phytotoxin that is required for full virulence (Bignell *et al.*, 2010a). Continued genome mining and functional analysis of biosynthetic gene clusters for small molecules and system-wide analyses of regulatory genes that control virulence will advance the understanding of pathogenesis by scab-causing streptomycetes even further and yield new methods of controlling this disease.

Plant endophytic streptomycetes

Endophytic actinobacteria have been isolated from a wide variety of plants and the most frequently isolated species belong to the genera *Microbispora*, *Nocardia*, *Micromonospora* and *Streptomyces*, the last of which is the by far the most abundantly observed (Sardi *et al.*, 1992; Taechowisan *et al.*, 2003). Endophytic and plant pathogenic *Streptomyces* species have similar life cycles in that they both colonize plant roots and ultimately invade the plant host. However, unlike pathogenic streptomycetes, endophytic species persist inside the plant host for long periods of time without causing observable disease symptoms and lack known virulence determinants common to phytopathogenic *Streptomyces* spp. (Coombs & Franco, 2003a, b). Endophytic *Streptomyces* bacteria are not simply plant commensals, but confer beneficial traits to their hosts that primarily fall into two categories: growth promotion and protection from phytopathogens.

Endophytic streptomycetes may enhance the growth of their plant host by the production of auxin, which is a plant hormone important for root growth and development (Coombs *et al.*, 2004; Overvoorde *et al.*, 2010).

Auxin production is not restricted to endophytes, but is widespread in the genus *Streptomyces* and other soil bacteria and probably reflects the success of these organisms in the rhizosphere (Manulis *et al.*, 1994; Patten & Glick, 1996). In addition to production of important plant hormones, endophytic *Streptomyces* spp. are capable of increasing nutrient assimilation by their plant host. For example, endophytic colonization of the pea plant *Pisum sativum* with the endophyte *Streptomyces lydicus* increases the frequency of root nodulation by *Rhizobium* spp., resulting in increased iron and molybdenum assimilation and more robust growth (Tokala *et al.*, 2002).

It is well accepted that members of the genus *Streptomyces* are prolific producers of antimicrobial compounds, and endophytic streptomycetes are no exception. Numerous endophytic *Streptomyces* isolates inhibit the growth of fungal phytopathogens *in vitro* and *in planta*, and this antibiosis has been proposed to be one of the mechanisms by which endophytes suppress plant diseases (Sardi *et al.*, 1992; Coombs & Franco, 2003a; Taechowisan *et al.*, 2003; Franco *et al.*, 2007). In addition to antibiosis as a means of resistance to pathogens, endophytic actinobacteria induce plant defence pathways in the model plant *Arabidopsis thaliana*; this systemic induction of plant defence pathways is thought to serve as a primer for defence and allows the plant host to respond more quickly to pathogen attack (Conn *et al.*, 2008).

The properties of endophytic *Streptomyces* spp. make them attractive biocontrol agents. Indeed, experiments both in the greenhouse and in experimental farm plots have demonstrated that endophytic streptomycetes increase the yield of wheat and decrease the incidence of

disease caused by *Gaeumannomyces graminis* (Coombs *et al.*, 2004; Franco *et al.*, 2007). The documented agricultural usefulness of endophytic *Streptomyces* species will no doubt stimulate future research to better understand the complex relationship between these *Streptomyces* symbionts and their plant hosts. In an age where our future food security is a serious concern, there is significant interest in understanding and promoting the beneficial bacterial interactions within the plant rhizosphere to increase crop production and decrease the incidence of disease (Mendes *et al.*, 2011).

Associations of *Streptomyces* species with fungi

Fungi and streptomycetes are abundant and diverse within the soil, but little is known about specific symbiotic interactions. Because many *Streptomyces* spp. produce secondary metabolites with antifungal properties or secrete chitinolytic enzymes, they are generally considered antagonists of both plant pathogenic and saprophytic soil fungi (Reddy & Satyanarayana, 2006; Lehr *et al.*, 2007; Tarkka & Hampp, 2008). However, some cases have been described in which streptomycetes promote the growth of rhizosphere fungi, notably those that are involved in forming ectomycorrhizae with plants (Maier *et al.*, 2004; Schrey & Tarkka, 2008; Hampp & Tarkka, 2009). Specifically, *Streptomyces* sp. AcH 505 produces the compound auxofuran, which promotes mycelial growth in the fly agaric (*Amanita muscaria*), an important mycorrhizal fungus of Norway spruce (Riedlinger *et al.*, 2006). Co-cultivation of *A. muscaria* with *Streptomyces* AcH 505 stimulated the

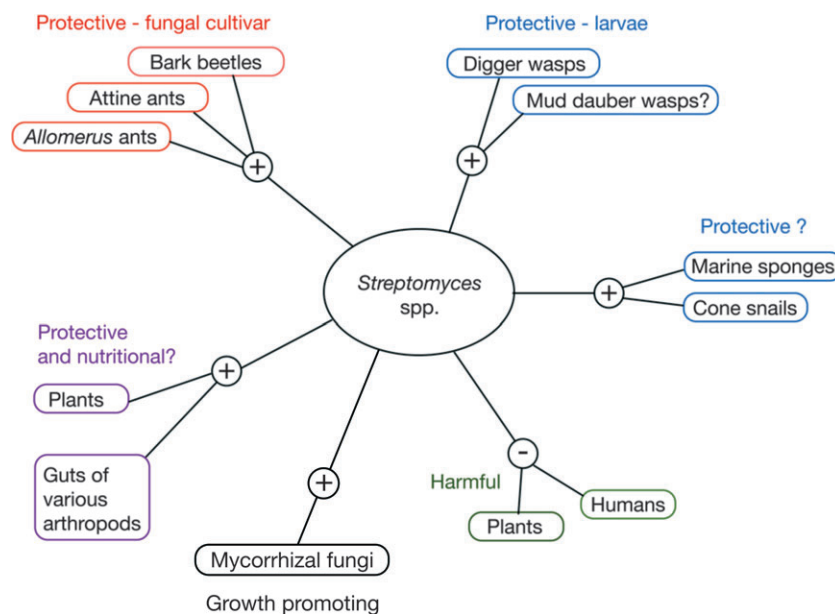


Fig. 7. The positive and negative interactions of *Streptomyces* spp. with eukaryotic organisms.

production of auxofuran by the streptomycete while suppressing the biosynthesis of the antifungal compound WS-5995 B, a potent inhibitor of plant pathogenic fungi. In *A. muscaria*, on the other hand, the interaction with AcH 505 strongly influenced the growth pattern, cytoskeleton structure and gene expression levels (Schrey *et al.*, 2005, 2007). Several other streptomycetes isolated from Norway spruce also promoted the growth of the mycorrhizal fungus, but did not affect plant pathogenic fungi (Maier *et al.*, 2004). These examples illustrate that soil streptomycetes can have growth-promoting as well as inhibiting effects on fungal growth (Tarkka *et al.*, 2009), and it seems likely that many other interactions between streptomycetes and rhizosphere fungi have yet to be uncovered.

Conclusions and future perspectives

In this review, we have attempted to summarize the current knowledge on the role of *Streptomyces* bacteria as symbionts and we hope that this will stimulate further research in this area (Fig. 7). The benefits to humans are obvious, both in terms of novel antibiotics and other secondary metabolites that could be used in human medicine and in promoting plant growth and preventing disease. Although much of the future research in this area is likely to be focused on these more applied aspects, it should also be a pivotal aim to understand the biological functions of the interactions between *Streptomyces* spp. and their host organisms. It is clear that symbioses between streptomycetes and eukaryotic organisms are important and most probably widespread. The diversity of these interactions likely played a major role in the evolution of their incredible antibiotic biosynthetic capabilities. We will need to understand and continue exploiting these capabilities if we are to stay ahead in the war against drug-resistant, pathogenic bacteria.

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