MINI-REVIEW

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Insights into the microbial world associated with ants

Abstract Insects are among the most successful animals of the world in terms of species richness as well as abundance. Their biomass exceeds that of mammals by far. Among insects, ants are of particular interest not only because of their enormous ecological role in many terrestrial ecosystems, but also because they have developed an impressive behavioural repertoire. In fact, a key feature of the evolutionary success of ants is their ability to form complex societies with division of labour among individuals in a colony belonging to different castes such as workers and soldiers. In addition to these complex social interactions of ants, they have shown an extraordinary capacity to build up close associations with other organisms such as other insects, plants, fungi and bacteria. In the present review we attempt to provide an overview of the various symbiotic interactions that ants have developed with microorganisms.

Keywords Symbiosis · Ants · *Blochmannia* · *Bartonella* *Wolbachia* · *Tetraponera* · *Camponotus* · *Atta* Bacteriocyte · Actinomycete

What is so special about ants?

Eusocial insects, comprising ants, bees, wasps and termites, have attracted a lot of attention from scientists due to their social organization and evolutionary success in terms of species richness and their extraordinary abundance in tropical regions. Social insects and especially ants have managed to expand not only into

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tropical areas but also to arctic regions and other extreme habitats like deserts. All known ant and termite species are eusocial and are organized in colonies with division of labour and sophisticated chemical communication. Eusociality is characterized by overlapping generations within a nest, brood care and the separation into a reproductive caste and sterile workers (Hölldobler and Wilson 1990). Colonies as a whole are long-lived, i.e. ant or termite queens may live for up to ~20 years in contrast to the short-lived workers (Hölldobler and Wilson 1990). Colonies may comprise several million individuals that provide a very stable environment concerning temperature, CO_2-levels and humidity within nests, parameters which are often regulated by the workers. The formation of castes, i.e. individuals that differ in either reproductive status or morphology in spite of an identical genetic background, has several important consequences not only for colony organization but also for associated microorganisms. The queen is most often the only reproductive individual in the colony and thus microorganisms residing in eggs can only be transmitted vertically by this single individuum. However, due to close interactions between individuals in the colony, e.g. food exchange between animals via trophollaxis, transfer of extracellular microorganisms between colony members may also occur.

Blochmannia, the obligate intracellular endosymbiont of carpenter ants

The first endosymbiosis of bacteria with animals ever described was that of intracellular bacteria with carpenter ants (*Camponotus ligniperdus*) (Blochmann 1892; Buchner 1965). The genus *Camponotus* is the most species-rich ant genus and currently comprises more than 1,000 described species (Hölldobler and Wilson 1990). Recent surveys demonstrated that each *Camponotus* species and a few closely related genera investigated so far carry these bacteria (Buchner 1965; Sameshima et al. 1999; Sauer et al. 2000; Schröder et al. 1996; Degnan

et al. 2004). The presence of the bacteria only in certain systematic groups of ants allows a rough estimate of the maximal age of this symbiosis which is around 50–70 million years. In honour of its discoverer these ant endosymbionts were recently named *Candidatus* Blochmannia (Sauer et al. 2000).

Related endosymbioses are very widespread among insects (Buchner 1965; Dasch et al. 1984). Analysis of the 16S rDNA sequences of the ant endosymbionts revealed that they belong to the γ3-subgroup of *Proteobacteria* and should be classified within the *Enterobacteriaceae* together with endosymbiotic bacteria of other insects such as *Buchnera aphidicola* in aphids (Baumann et al. 1995; Belda et al. 2005; Canback et al. 2004; Gil et al. 2004; Sauer et al. 2000; Wernegreen 2002). Thus, these endosymbionts have a common ancestor with the genera *Escherichia, Salmonella* and *Yersinia*, many members of which are pathogens for animals or man (Boursaux-Eude and Gross 2000; Goebel and Gross 2001). Comparison of the phylogenetic relationships of these endosymbionts with those of their host animals reveals co-evolution of the two partners indicating a strict maternal transmission of the bacteria (Baumann et al. 1995; Chen et al. 1999; Funk et al. 2000; Sauer et al. 2000; Degnan et al. 2004).

In ants the endosymbionts are located in specialized cells, the so-called bacteriocytes, which are intercalated between normal epithelial cells of the midgut and in the ovaries of workers and queens (Fig. 1) (Buchner 1965;

Dasch et al. 1984; Sauer et al. 2002; Schröder et al. 1996). The bacteria are obligate intracellular and thrive free in the cytosol of the host cells. Interestingly, *Blochmannia* tends to disappear from the midgut bacteriocytes with increasing age of the animals but not from the ovaries. This indicates that endosymbiosis is of particular relevance in the earlier phases of the animal's life-cycle, but the bacteria may become dispensable with increasing age of the adult animals when transmission to the progeny is guaranteed (Wolschin et al. 2004). In fact, treatment of a *C. floridanus* queen with antibiotics resulted in a defective reproduction with the brood arrested during early phases of embryogenesis (Wolschin et al. 2004).

Bacteriocyte symbioses are also found in many other insects such as in aphids with *B. aphidicola* (Buchner 1965; Dasch et al. 1984). Such symbioses are generally considered to have a nutritional basis with the endosymbionts providing essential food supplements to their host animals. In fact, the host animals in such symbioses generally feed on a very specialized diet such as plant sap or blood devoid of essential compounds (Baumann et al. 1995; Douglas 1998; Nogge 1982). The concept of food supplementation of the host animals by such endosymbionts is now confirmed by recent genomic data (Akman et al. 2002; Gil et al. 2003; Shigenobu et al. 2000; Tamas et al. 2003; Wernegreen 2002; Zientz et al. 2001, 2004). Modern genome techniques were a major breakthrough, since they allowed a significant cultiva-

Fig. 1 Electron micrograph of a bacteria-filled bacteriocyte embedded between midgut enterocytes of a worker of *C. floridanus*. Magnification ~2,000-fold
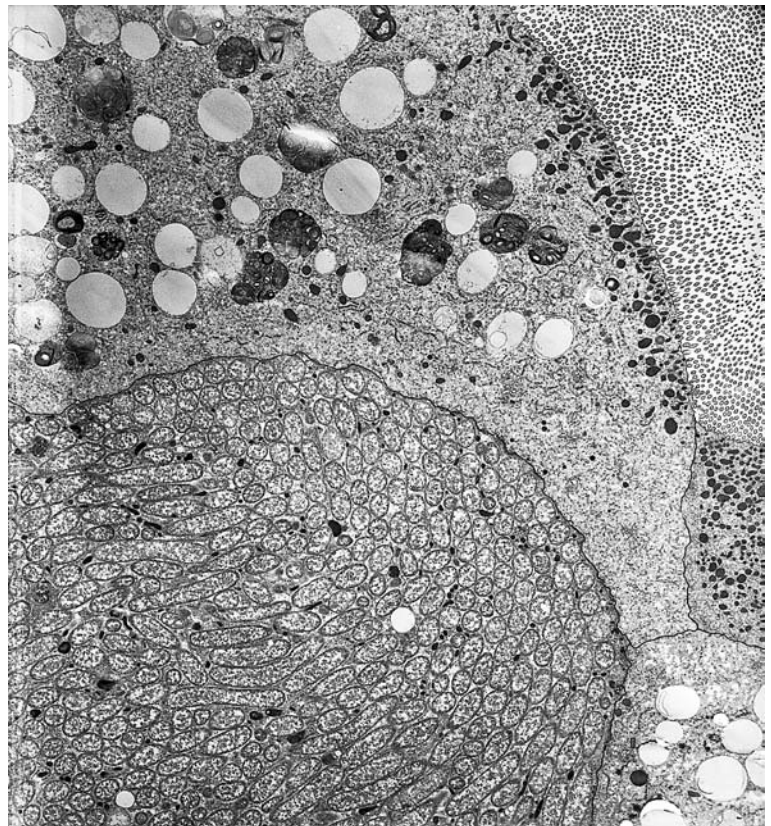
Fig. 2 Hypothetical metabolic interactions in the *Blochmannia–Camponotus* symbiosis as deduced from the *B. floridanus* genome sequence. Only several relevant pathways are shown. Compounds probably imported by *Blochmannia* are shown in *red*, compounds likely to be transferred from *Blochmannia* to the bacteriocyte are shown in *blue*. For further details see the text
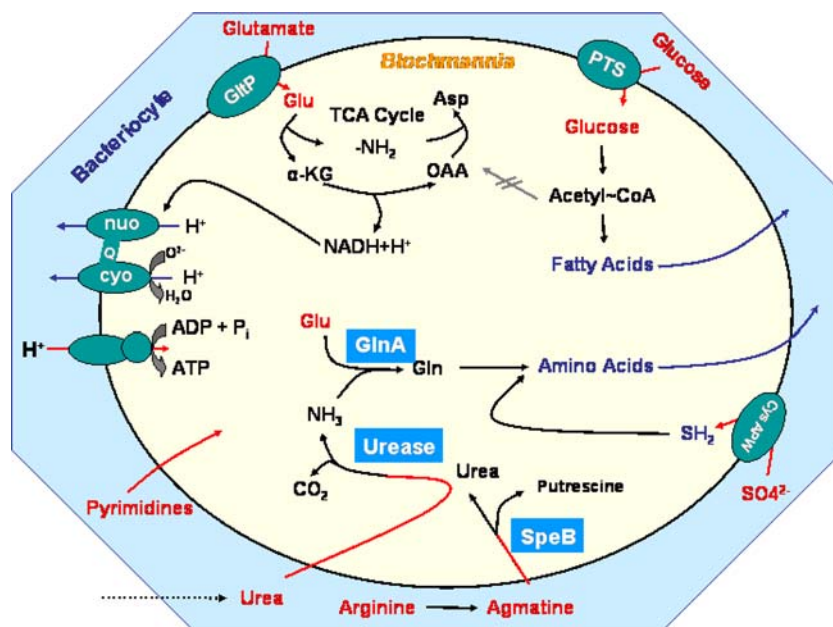
tion-independent genetic analysis of these uncultivated microorganisms. The analysis of the gene content of several endosymbiont genomes reveals some common trends. The endosymbionts have extremely reduced genome sizes of about 450–800 Kbp with a corresponding low gene content and an extremely high AT-content of about 75% (Gil et al. 2002, 2003; Shigenobu et al. 2000; Tamas et al. 2002; van Ham et al. 2003; Wernegreen 2002).

Assignment of the *Blochmannia* encoded genes to the functional categories classified in the COG database shows that with the exception of translation-relevant factors members of all other functional categories are strongly reduced. This is in particular prominent for factors involved in transcription regulation, signal transduction, DNA repair and recombination. Surprisingly, *Blochmannia* lacks the replication initiation protein DnaA indicating a direct control of bacterial replication by the host organisms. In fact, in ants replication of the bacteria takes place only during specific developmental phases of the host, i.e. during pupation and in young adults directly after eclosion (Wolschin et al. 2004).

Virtually all *Blochmannia* genes have their closest orthologs within the family *Enterbacteriaceae* allowing a detailed characterization of their metabolic potential in comparison to *E. coli* (Zientz et al. 2004). While a nutritional role of the bacteriocyte symbioses is obvious in other cases, the situation in the ant endosymbiosis is more complex, since carpenter ants are known to be omnivorous. However, the genome sequence suggests that endosymbiosis with *Blochmannia* also has a nutritional basis and mainly may contribute to nitrogen, sulphur and lipid metabolism of the host animal (Fig. 2). The *Blochmannia* genome sequence demonstrates the presence of all biosynthetic pathways required for the synthesis of amino acids essential to the animals (with the exception of arginine), while several biosynthesis pathways required for the synthesis of non-essential amino acids have been lost. Moreover, *B. floridanus* retains a complete pathway to reduce sulphate and to synthesize cysteine. Since insects are not able to reduce sulphur compounds, this bacterial pathway may be very important for the animals. The genome sequence suggests that *Blochmannia* has an obligate aerobic metabolism and can oxidize glucose imported by a phosphotransferase import system (PTS) through glycolysis. However, acetyl-CoA cannot be further processed via the citric acid cycle, since in *B. floridanus* this cycle is present only partially lacking the C2-fixing steps. It starts with α-oxoglutarate which can be oxidized to oxaloacetate as a final product, thus conserving the energy-yielding steps. Acetyl-CoA should therefore be utilized only for the biosynthesis of fatty acids, which may have an important function in this endosymbiosis. The presence of the high affinity transport system GltP for glutamate suggests that glutamate is imported from the host cell and is the source of α-oxoglutarate required for the truncated citric acid cycle. *Blochmannia* is not capable of gluconeogenesis and should utilize imported sugars for the biosynthesis of sugar-containing cell components such as the mureine or lipopolysaccharide. A prominent feature of *B. floridanus* is the presence of a urease which may help the animals to recycle nitrogen compounds in developmental phases such as metamorphosis in which the animals do not take up external nutrients.

Compared with their free living relatives, *Blochmannia* shows a remarkably increased evolution rate possibly resulting in the corrosion of its genetic material (Fry and Wernegreen 2005). This may be due to frequent bottlenecks occurring during maternal transmission of only a

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relatively small number of the bacterial population resulting in a low effective population size as suggested previously for *Buchnera* (Mira and Moran 2002). The accumulation of such mutations is also favoured by the virtual lack of DNA repair mechanisms and of horizontal gene transfer events. The evolutionary consequences of such a presumed erosion of the genetic material for the symbiosis are unknown; however, it may be a threat to symbiosis in the long-term.

In conclusion, the animals provide the bacteria a safe and constant environment, which has caused a very strong reduction in the metabolic capabilities, signal transduction and gene regulatory repertoire of the bacteria leaving only symbiosis-relevant pathways. Moreover, the intracellular environment may have allowed the bacteria even to reduce their structural integrity, e.g. by the reduction of cell wall structures such as the lipopolysaccharide which otherwise are important to protect the bacteria from detrimental environmental cues. However, it appears that in the long-lasting *Blochmannia-Camponotus* symbiosis the evolutionary forces led to a highly adapted beneficial co-existence between the bacteria and their host which is mainly based on metabolite exchange between the two partners.

Wolbachia infection in ants

Wolbachia infections in ants are widespread (Keller et al. 2001; Van Borm et al. 2002a; Wenseleers et al. 1998, 2002). *Wolbachia* is an obligate intracellular α-*Proteobacterium* belonging to the Rickettsiae which is transmitted maternally via the egg cytoplasm, just like its relatives, the mitochondria. In many arthropods *Wolbachia* is able to manipulate the host's reproduction in different ways to enhance its transmission to the progeny. All these mechanisms finally lead to a higher proportion of *Wolbachia*-infected females in comparison to uninfected females or males (Charlat et al. 2003; McGraw and O'Neill 2004; Stouthamer et al. 1999). To achieve this goal, dependent on the group of arthropods, *Wolbachia* is known to cause feminization of males (Rigaud et al. 2001), male killing (MK) (Fialho and Stevens 2000; Hurst et al. 2000), induction of parthenogenesis or differential killing of uninfected females or females carrying a different *Wolbachia* strain via cytoplasmic incompatibility (CI) (Stouthamer et al. 1999). However, male animals as well as sterile female workers of social insect colonies are dead ends for its propagation, raising the question of whether and how *Wolbachia* manipulates the reproduction of social insect colonies in comparison to solitary hymenoptera or other arthropods.

Initially most studies of consequences of *Wolbachia* infections in ants concentrated on induced parthenogenesis, since, although the majority of ants produce females via fertilized eggs, a number of ant species are known that reproduce via thelytokous eggs, i.e. unfertilized diploid eggs. However, in none of the respective ant species examined an infection with *Wolbachia* could be detected, indicating that *Wolbachia* does not induce parthenogenesis in social insects (Wenseleers and Billen 2000).

Interestingly, in ant colonies not all castes and life-history stages show the same level of infection. Adult workers and males often have a lower infection level than female reproductives or worker brood (Van Borm et al. 2002a; Wenseleers et al. 2002). Loss of infections within colonies when workers become older and lower infection rates in workers compared to sexuals point towards an adaptive mechanism: *Wolbachia*-infected colonies have reduced fitness as they produce a significantly lower number of sexuals than uninfected colonies or colonies with a lower parasite load (Wenseleers et al. 2002). Therefore, not only is the colony fitness reduced but also the success of transmission for the parasite and thus the interests of *Wolbachia* and the ant colony are in line and both would benefit from clearance of the infection in the worker caste. A loss of *Wolbachia* infection in colonies of invasive ant species in populations in the introduced range has also been observed (Shoemaker et al. 2000; Reuter et al. 2005), possibly facilitating their success in new habitats. The absence or low level of infection may be due either to drift (founder effect) or to different selection pressures in the new habitat in comparison to the native range (Reuter et al. 2005).

Several authors have tried to detect evidence for CI or MK in natural populations of ants. Presence of CI can be tested on the population level comprising numerous colonies: if CI occurs in social insects, then the frequency of uninfected colonies in a population with high parasite prevalence should be lower than the frequency of uninfected virgin queens produced in an infected colony, which happens to a certain percentage as *Wolbachia* may be lost due to segregation. Incompatible matings between uninfected virgin queens and infected males should eliminate most uninfected queens from the population during colony founding as they would be unable to produce any workers. Wenseleers et al. (2002) and Van Borm et al. (2002a) have indeed found lower rates of uninfected colonies than uninfected virgin queens produced in the population, but whether this is really a result of CI still remains unclear since the observed pattern was still within the limits of the sampling error.

Van Borm et al. (2002a) found significantly higher rates of uninfected males in comparison to uninfected virgin queens within colonies of *Acromyrmex* leaf-cutter ants. This finding could theoretically be explained by both, MK and CI. In the more probable MK scenario infected males do not survive but infected females do and thus, if transmission to the offspring is imperfect, the level of uninfected males is expected to be significantly higher.

Horizontal transmission of *Wolbachia* may occur very frequently in ants as suggested by the sequence similarity of strains in three ant genera *Linepithema*, *Acromyrmex* and *Solenopsis* located in Latin America (Reuter et al. 2005). Dedeine et al. (2005) discuss social parasitism between closely related species of the fire ant *Solenopsis* as a possible mechanism of horizontal

transfer that would be unique to social insects, suggested by identical strains of *Wolbachia* found in the parasitic as well as the host species. The parasite species may acquire new strains of *Wolbachia* through close contact with workers of the host species that feed the brood of the parasite.

Future work is required to clarify the role of *Wolbachia* for the biology of social insects. To date experimental studies on the reproductive consequences of *Wolbachia* infections in ants are still insufficient, possibly due to the long generation times of ants as well as the difficulties in getting sexuals to mate under laboratory conditions. Nonetheless, these studies should be carried out since different castes within a social insect colony pose a special problem to *Wolbachia* as described above and may thus enhance our understanding of the evolution and regulation of the pathogenicity of this widespread bacterium.

Extracellular gut endosymbionts of ants

For a long time the nutritional basis for the high abundance and diversity of ants especially in canopies of tropical rain forests was a mystery. Only recently studies on the trophic level of ants using stable isotopes revealed that they mainly utilize nitrogen-poor plant sap or honeydew from trophobiosis with aphids and other insects instead of being mainly predators as had been postulated before (Blüthgen et al. 2003; Davidson et al. 2003). However, the low concentration of physiologically usable nitrogen compounds in this diet should limit colony growth severely (Davidson et al. 2003). An alternative source of nitrogen for those ants could derive from symbiotic bacteria in the ants' gut that recycle waste nitrogen or fix atmospheric dinitrogen similar to what is known from termites and their gut microflora (Brune and Friedrich 2000). Recently, a special gut pouch was described in the *nigra* group of the ant genus *Tetraponera* (Pseudomyrmecinae), whereas such a pouch was missing in the other major *Tetraponera* group examined (the *allaborans* group). Histological studies revealed that this pouch-like structure between the midgut and the intestine was filled with a dense bacterial mass (Van Borm et al. 2002b). First analysis of the bacteria residing in this gut pouch revealed that the pouch is filled with relatives of different root-nodule-associated α-, β- and γ-Proteobacteria as well as Flavobacteria, and a role of these bacteria in nitrogen metabolism of the animals was suggested (Van Borm et al. 2002b). In a more extensive study comprising ants of three species groups of *Tetraponera* it has been shown that each group of host species harbours a specific microflora. In all *Tetraponera* species of the *nigra* group a monophyletic cluster of endosymbionts related to the order Rhizobiales was detected. Phylogenetically, these bacteria are closely related with *Bartonella* (Stoll 2005). Interestingly, in *Tetraponera* colonies of the *allaborans* group lacking the pouch organ other group-specific

symbionts were detected with close affiliation to the γ-Proteobacterium *Sodalis glossinidius* (Stoll 2005), a secondary symbiont of tsetse flies (Dale and Maudlin 1999). In *T. pilosa*, which forms a monotypic group within the oriental *Tetraponera*, *Pantoea agglomerans* was found, a γ-Proteobacterium that is known to occur in termites and to fix nitrogen from the air (Ohkuma and Kudo 1996). In spite of the different phylogenetic affiliation of the bacteria found in the three *Tetraponera* species groups, a feature common to these bacteria is the presence of an orthologous gene encoding a structural subunit of the dinitrogenase reductase *nifH* (Stoll 2005). This suggests a function of the bacterial microflora of the ants in nitrogen fixation and may explain why these animals are so successful in their arboreal habitats.

The tripartite symbiosis of leaf-cutter ants, their fungal cultivars and an actinomycete

More than 50 million years ago a mutually beneficent symbiosis between the New World fungus-growing ants (Hymenoptera: Formicidae: Attini) and their fungi (Agaricales: mostly Lepiotaceae: Luecocoprineae) evolved (Currie 2001). In this symbiosis the ants cultivate a fungus. The fungus is the predominant food source of the ants and they became dependent on the successful cultivation of their crop. For larvae and the queen the fungus is the sole food resource, while workers may supplement this diet by feeding on plant sap. Similar developments occurred in the Old World in the case of termites and their fungus gardens, as well as in the cosmopolitan fungus-growing beetles (Aanen et al. 2002; Jones and Blackwell 1998). Together these animals play dominant roles in many ecosystems, but they can also be considered to be relevant agricultural or forest pests.

In ants, the ability to cultivate fungi has evolved only once giving rise to more than 200 known fungus-growing species. These animals can be organized in small colonies consisting of a few dozens of animals in the case of the so-called lower attines up to very large colonies in the case of the most phylogenetically derived leaf-cutter ants of the genus *Atta* and *Acromyrmex* which can be composed of several millions of animals. The symbiotic fungus of attines is transmitted vertically leading to a co-evolutionary history between fungus growers and their cultivated symbionts (Hinkle et al. 1994). During nuptial flight, young foundress queens carry a small boulder of the fungus within a special cavity within their mouthparts, the infrabuccal pocket. The foundress queen herself takes care of the cultivation of the fungus until the appearance of the first workers which then take over the little fungus garden and start to forage for garden substrate. Fungus gardens are monocultures of single fungus-strains, which minimize conflicts and competition that may arise between different strains of the symbiotic fungi. Interestingly, the domesticated fungi of one nest are able to actively reject mycelial components from neighbouring colonies contributing to

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the strictly vertical transmission of fungi (Poulsen and Boomsma 2005). Additionally, ants prevent the symbiotic fungi from sexual reproduction, thus preventing recombination and saving the energetic costs of fruiting. As can be imagined easily, the fungus gardens as ancient asexual organisms are under permanent threat to be parasitized by microorganisms or devastated by predatory animals such as nematodes.

In fact, recent work indicates that a major threat to the *Atta* fungus gardens is the invasion by parasitic fungi, especially by the hyphomycete *Escovopsis* (anamorph allied with Hypocreales: Ascomycota). *Escovopsis* was found to be the most common non-mutualistic fungus present in fungus gardens and was shown to be a specialized pathogen of the fungus cultivars (Currie et al. 1999a). Phylogenetic analysis also indicates a long tripartite co-evolution of *Escovopsis* with the ants and their fungal gardens (Currie et al. 2003b).

This raises the question of how pathogens are controlled by ants and their mutualistic fungi. It was observed that all fungus-cultivating ants investigated so far are associated with a "waxy bloom" on certain areas of their cuticle which was previously thought to be a cuticular exudate. However, more recent data showed that this material is composed of filamentous bacteria (Currie et al. 1999b). The bacteria are apparently absent in males. The 16S rDNA sequence identified these bacteria to be classified within the *Pseudonocardiaceae* (Currie et al. 2003a). Actinomycetes are well-known producers of secondary metabolites including many important antibiotics. A phylogenetic analysis of the bacteria revealed a co-speciation between the animals and their bacteria indicating a long-lasting vertical transmission of the bacteria. Bioassays have shown that the bacteria on the ants' cuticle play a role in maintaining an acceptable equilibrium between the pathogens and the fungal cultivars possibly by antibiotics production. The antimicrobial compounds produced by the actinomycete are effective exclusively against the pathogenic fungus *Escovopsis*. So far, the chemical nature of these antimicrobials is not known. Moreover, in broth culture bioassays evidence was obtained which suggests that the presence of a filtrate from a bacterial culture has a positive effect on biomass production by the fungal cultivar of the phylogenetically basal attine genus *Apterostigma*. Accordingly, bacteria may produce compounds which are growth-promoting for the fungal cultivars in addition to antimicrobial agents, e.g. vitamins or amino acids. In exchange, the animals propagate the bacteria, may contribute to their nutrition and provide them a unique niche and competition-free space in respect to other microorganisms.

Conclusions

The above-described examples provide first insights into the plentitude of ant–microbe interactions. However, although ants are so abundant and play an important role in many terrestrial ecosystems, so far only very limited knowledge has been gathered about microbes associated with ants. Since ants are characterized by complex social interactions, the symbioses with microorganisms can play eminent roles not only on the level of the individual but also on the colony level. Moreover, ants have developed a vast capability to adapt to entirely different ecosystems and it is likely that microbial companions play a major role in such adaptations. The characterization of the partnership of microorganisms with ants may therefore provide an entirely new comprehension of the evolution of symbiotic and parasitic microbe–host interactions and their ecological relevance.

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