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FUNGUS-GROWING INSECTS HOST A CONVERGENT MICROBIOME WITH FUNCTIONAL SIMILARITIES TO OTHER LIGNOCELLULOSE-FEEDING INSECTS

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"Do not think that time simply flies away. Do not understand 'flying' as the only function of time. If time simply flew away, a separation would exist between you and time. So, if you understand time as only passing, then you do not understand the time being. To grasp this truly, every being that exist in the entire world is linked together as moments in time, and at the same time they exist as individual moments of time." (Dōgen Zenji, Uji)

As vezes penso que não vi o caminho que percorri, agora que estou chegando ao final. Posso não ter lembranças de muitos detalhes, mas isso não significa que não observei esse trajeto; mais importante que observar é entender as mudanças. Eu mudei a forma pela qual entendo a vida, e isso só é possível devido às experiências associadas a esse percurso. Se me perguntassem pelo resultado final, diria que aprendi muito. Aprendi com pessoas, ouvindo e explicando; aprendi com (muitos) livros e artigos; aprendi com experiências, expectativas e medos. O aprendizado é uma consequência do meio no qual estamos imersos, e a todos os que participaram desse processo, eu gostaria de agradecer:

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"Look again at that dot. That's here. That's home. That's us."

Carl Sagan

RESUMO

Insetos cultivadores de fungos (formigas, cupins e besouros) evoluíram de forma independente uma associação simbionte com fungos que, ao metabolizar biomassa vegetal recalcitrante, produzem nutrientes disponíveis para seu hospedeiro. Esses sistemas de fungicultura também abrigam microbiomas bacterianos que apresentam importantes impactos fisiológicos na biologia do inseto fungicultor. Nesse trabalho, foram explorados os padrões de convergência funcional da microbiota associada a sistemas de fungicultura. Com o intuito de expandir a distribuição geográfica de microbiomas associados a sistemas de fungicultura, metagenomas de comunidades bacterianas de *Mycocepurus goeldii* (Attini basal) e *Atta sexdens rubropilosa* (Attini derivada, também denominada formiga-cortadeira de folhas) foram sequenciados e anotados. Tais amostras constituem os primeiros microbiomas de formigas Attini da América do Sul. Os gêneros *Pseudomonas, Pantoea, Rhizobium, Enterobacter, Achromobacter*, *Stenotrophomonas* e *Serratia* foram os mais abundantes na comunidade bacteriana associada ao jardim de fungo de *A. sexdens rubropilosa. Pseudomonas* também foi o gênero encontrado em maior abundância na comunidade bacteriana de *M. goeldii*, seguido de *Dysgonomonas, Bacteroides*, *Parabacteroides*, *Prevotella, Comamonas* e *Burkholderia*. A fim de explorar o perfil funcional da comunidade bacteriana, foram realizadas comparações entre microbiomas de Attini basais e derivadas; de insetos fungicultores; de insetos fungicultores e trato intestinal de insetos cuja alimentação é baseada em lignocelulose. As análises comparativas revelaram que os microbiomas associados a insetos fungicultores apresentam uma evidente convergência funcional e taxonômica. É possível verificar a existência de similaridades funcionais entre microbiomas de insetos fungicultores e do trato intestinal de insetos herbívoros, principalmente em relação às rotas envolvidas no metabolismo de carboidratos, aminoácidos, compostos aromáticos, cofatores e vitaminas. No entanto, diferenças taxonômicas notáveis podem ser observadas entre microbiomas de insetos fungicultores e trato intestinal de insetos herbívoros, fornecendo evidências adicionais para a convergência funcional na comunidade bacteriana associada a insetos cultivadores de fungos.

Palavras-chave: Convergência funcional. Associação hospedeiro-microbioma. Fungicultura. Attini. Macrotermitinae. Scolytinae. Metagenoma. CAZy.

ABSTRACT

Fungus-growing insects (ants, termites, and beetles) independently evolved a symbiotic association with fungi that metabolize recalcitrant plant biomass, producing nutrients available to the insect host. These fungicultural systems also harbor bacterial microbiota of important physiological impacts for the host life style. Here, we explore convergence patterns of the microbiota associated with fungiculture systems. For expanding the geographic distribution of microbiomes fungiculture systems available, we sequenced and annotated metagenomes of bacterial communities from *Mycocepurus goeldii* (lower Attini ant) and *Atta sexdens rubropilosa* (higher Attini, a leaf-cutter ant), the first attine ants' microbiomes from South America. *Pseudomonas, Pantoea, Rhizobium, Enterobacter, Achromobacter, Stenotrophomonas* and *Serratia* were the most abundant genera in the bacterial community of *A. sexdens rubropilosa* fungus garden. Similarly, *Pseudomonas* was also the most abundant genus in the bacterial community of *M. goeldii* fungus garden, followed by *Dysgonomonas*, *Bacteroides*, *Parabacteroides*, *Prevotella, Comamonas* and *Burkholderia*. For metabolic profiling, these microbiomes were included in comparisons of several levels: between lower and higher attines, among fungus-growing insects, and between fungus-growing and non-fungus-growing insects. Comparative analysis of fungus-growing insects associated microbiomes support remarkable functional and taxonomic similarities, pointing to convergence in bacterial communities. Metabolic parallels may be found among microbiomes from fungus-growing insects and other lignocellulose-feeding insects, particularly for pathways involved with the metabolism of carbohydrates, amino acids, aromatic compounds, cofactors and vitamins. However, there are substantial taxonomic differences between microbiomes from fungiculture systems and herbivorous insects' gut, giving further evidence for the functional convergence in bacterial microbiota associated with fungus-growing insects.

Keywords: Functional convergence. Host-microbiome association. Fungiculture. Attini. Macrotermitinae. Scolytinae. Metagenome. CAZy

CONTENTS

Fungus-growing insects host a convergent microbiome with functional similarities to other lignocellulose-feeding insects

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1 Introduction

Host-associated microbial communities comprise complex ecological systems, shaped by several stochastic and selective forces.[1,2] Most of microbial functional traits are not phylogenetically exclusive, and different microorganisms could occupy similar functional niches within the host.[2,3] Thus, the assembly of functionally equivalent species within a niche could be an outcome of natural selection acting mainly on microbial functional traits.[2] Identifying the functional repertoire of a microbiome could potentially predict ecological parameters and evolutive processes shaping the microbiota composition.[2,4] Also, the functional capabilities of host-associated microbial communities provide valuable information on how the interactions among community members influence the host's physiology.[5-7] Interactive host-microbiota metabolism could be considered a key determinant of the host life style and fitness over an evolutionary time scale.[6,8,9] The dynamics of this metabolic communication also affects the diversity, composition, and functional capacity of the associated microbiota, which is shaped by a series of interactions throughout the host life, including diet, physicochemical variations, and maturation phases.[9]

Only by considering the microbiota as a component of ecological and physiological processes, the nutritional ecology of insects can be explained.[6,10] Particularly for herbivorous insects, which may feed on recalcitrant and indigestible plant tissues, with low nutritional content and toxic compounds, the association with symbiotic microorganisms mediates the use of resources otherwise non-accessible to the host.[6,11] Fungus-growing insects are a noticeable example of insect-microbial symbiosis for exploring recalcitrant plant biomass. The active maintenance of fungus crops, also known as fungiculture, evolved independently in tree insect lineages:[12] ants in the tribe Attini (Hymenoptera: Formicidae: Myrmicinae), which are strict to the New World, occurring from the south of Argentina to the south of United States;[13] termites in the subfamily Macrotermitinae (Isoptera: Termitidae), which occur in the Old-World tropics, mainly in Africa and Asia;[14] and beetles in the subfamily Scolytinae (Coleoptera: Curculionidae), which are found in wet tropical forests.[15] These "fungus farmers" exhibit specific methods for fungus cultivation, harvesting and propagation (Fig. 1).[12]

The fungal lignocellulose-degrading capacity has been fundamental for the evolutionary success of the fungus-growing insects symbiosis.[16-18] The so-called lower attines (Fig. 1A)

cultivate basidiomycete fungal symbionts (mostly of the tribe Leucocoprinae) in diverse substrates. These fungi are not truly domesticated and are able to sustain a free live existence.[12,19-21] Higher attines (Fig 1B) cultivate a fully and highly domesticated basidiomycete fungal symbiont in the genus *Leucoagaricus,* which is unable of a free-living existence and apparently has been clonally dispersed through vertical transmission.[12,22-24] The ambrosia beetle fungal symbionts are usually the ascomycete genera *Ambrosiella* and *Raffaelea,* which are cultivated on the walls of galleries excavated in the xylem of trees, and comprise the only nutritional source for larvae and adults (Fig 1C).[18,25-27] Although bark beetles cannot be considered true "fungus farmers", they are commonly associated with ascomycetes in the genera *Ophiostoma*, *Ceratocystiopsis*, *Grosmannia*, and *Ceratocystis.* Bark beetles main nutritional source is phloem, but the fungal association may be related to improving nutrients availability and detoxification of host-defense metabolites.[27,28] Plant biomass degradation in fungus-growing termites' colonies (Fig 1D) depends on the enzymatic activity of a basidiomycete fungal symbiont in the genus *Termitomyces,* in combination with workers' gut microbiota.[17,29] *Termitomyces* grows within fungus combs, which are structures built from termite feces containing pre-digested plant material (processed by the termite and by the enzymatic activity of the gut microbiota) and asexual spores of *Termitomyces*.[17,30-32]

Fungus-growing insects also harbor bacterial microbiotas of important physiological consequences for the host life style. For example, some components of the bacterial microbiota from higher Attini ants are able to fix atmospheric nitrogen,[33] and the community also exhibit the metabolic capacity for lignocellulose degradation, aminoacids and vitamins biosynthesis.[34,35] Bacteria found in the galleries of ambrosia beetles are considered secondary symbionts,[36,37] while bacteria associated with bark beetles are able to produce antimicrobial compounds[38] and can degrade terpenes, which are employed as chemical defense released by conifers as a response to bark beetles attacking behavior.[39,40] In termite fungiculture, besides antimicrobial-producing bacteria which could suppress antagonistic fungus of the symbiosis,[41] the workers gut microbiome and the fungus comb microbiome aid in the comb continuous lignocellulose decomposition.[17,32] The metabolic potential of the microbiota associated with fungus-growing insects suggests that the bacterial community could be a usual and determinant characteristic of fungiculture systems.[42]

Despite differences in insect host geographic distribution and life history, bacterial microbiomes associated with fungus-growing insects exhibit a highly similar taxonomic composition, indicating that these communities could be an example of convergence of host-microbiome association.[42] Convergent evolution manifests as the independent evolution of similar traits or phenotypes (as physiological or morphological features of a microorganism) in multiple lineages.[43-45] The convergence of phenotypes provides remarkable evidence that particular environmental conditions probably select for specific traits - which represents similar evolutionary solutions.[46] When referring to convergent evolution of microbiotas, we are considering that a set of similar functions and/or taxonomic composition may be found in the microbiota associated to different and distantly related insect hosts. Here, we explore whether convergence patterns may be found in the microbiota associated with fungiculture systems. For expanding the geographic distribution of the microbiomes available, we included the first Attini microbiomes from South America. Our choice for adding microbiomes from the lower attini *Mycocepurus goeldii* and the higher attini *Atta sexdens rubropilosa* was based on the wide distribution of these species in Brazil.[47,48] We first described the taxonomic composition and the functional capacity of the bacterial microbiota from fungus gardens of both ant species. Then, we included these microbiomes in a broad comparison of microbiota functional profile in several levels: between lower and higher attini, among fungus-growing insects, and between fungus-growing and non-fungus-growing insects. Comparative analysis of the microbiomes support the bacterial microbiota taking place in plant material decomposition. Metabolic parallels may be found among microbiomes from fungus-growing insects and other lignocellulose-feeding insects, particularly for pathways involved with the metabolism of carbohydrates, amino acids, aromatic compounds, and cofactors and vitamins. Nevertheless, notable differences in taxonomic composition of microbiomes from fungiculture and insects' gut not only emphasize taxonomic similarities in fungiculture systems,[42] but also reinforce different host-microbiome associations as functionally similar environments. As such, fungiculture systems' microbiota comprise a remarkable example of functionally convergent bacterial community.

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