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Application of DNA sequence based taxon identification to the study of root associated fungal communities

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Zusammenfassung

Die Anwendung molekularer Methoden enthüllte die unerwartete Diversität Wurzel-assoziierter Pilze. Besonders die symbiotischen Pilzpartner fordern die Pilztaxonomie heraus, und nur durch Kombination molekularer und morphologischer Analysen ist es möglich, die bestehenden Klassifikationen zu verbessern. Arten sind elementare Einheiten der Biologie, und eine gut abgesicherte Taxonomie ist unerlässliche Grundlage für Biodiversitätsstudien und experimentelle Forschung. Diese scheinbar einfache Aufgabe wird durch die vergleichsweise geringe morphologische Differenzierung bei hoher phylogenetischer Diversität erschwert. Die nächste große Herausforderung ist die Schaffung von Datenbanken funktioneller Gene wurzelassoziierter Pilze, als Basis für metagenomische Studien der Wurzel-Pilz-Boden Interaktionen.

Summary

Molecular tools revealed unexpected diversity among different guilds of root associated fungi.

Particularly the symbiotic root associates constitute a major challenge for fungal taxonomy, a combined molecular and morphological approach is needed to improve the classification

Species are the basic units of biology, and a reliable, stable taxonomy of root associated fungi is needed as a basis of biodiversity studies and experimental research. This seemingly simple task is complicated by the fact that morphological differentiation is low compared to the deep phylogenetic diversity recently discovered in various groups of root associated fungi. The next major challenge is the establishment of databases of functional genes of root associated fungi, to explore the metagenomics of plant-fungus-soil interactions.

Keywords: Sebacinales, biodiversity, mycorrhiza, evolution, symbiosis

Introduction

Here, the term root associated fungi is used to denote a variety of guilds or functional groups of fungi, which overlap only in part with systematic respectively phylogenetic groups. **Mycorrhizal** fungi form intimate mutualistic interactions with plant roots, the mycorrhizal symbiosis is based on an exchange of mineral nutrients and carbohydrates between fungi and plants. Different types of mycorrhizal associations are distinguished. **Arbuscular Mycorrhiza (AM)**, which are exclusively formed by members of the Glomeromycota, are most universally distributed in the plant kingdom, and they are likely to have facilitated the colonisation of terrestrial habitats by plants from the beginning of land plant evolution. **Ectomycorrhiza (ECM)** is more specific to a selection of plant families, which however, include many of the dominant tree species of boreal, temperate, mediterranean and certain (sub-)tropical areas, e.g.. Pinaceae, Betulaceae, Fagaceae, Salicaceae, Dipterocarpaceae, eucalypts, certain Rosaceae and Fabaceae, and others. **Ericoid mycorrhiza** is specific to ericaceous host plants, typically ocurring in strongly Nitrogen limited soils, e.g. in heaths and bogs (SMITH and READ 2008). Mycorrhizal samples are often co-colonized by **mycorrhiza co-associated fungi** (URBAN et al. 2008). Ectomycorrhiza co-associated fungi include a diverse assemblage of ascomycetes with helotialean, hypocrealean and chaetothyrialean affinities, some of the former appear to be closely related to or identical with ericoid mycorrhizal fungi, and are also known as **Dark Septate Endophytes (DSE). Fungal root endophytes** are rather negatively defined, as are endophytes in general. They are neither regarded as mycorrhizal or pathogens and they do not cause recognizable symptoms in their host plants. They comprise a diverse and still incompletely known assemblage of fungi, some of them may have mutualistic properties, e.g. by conferring resistance against plant pathogens, some of them may simply be latent, asymptotic states of plant decomposers. The **rhizosphere**, a microhabitat defined by the activity of fine roots, is characterised by specific properties different from the bulk soil. In the depletion zone, the concentration of less diffusible mineral nutrients, particularly phosphates, is low, and the concentration of low molecular weight organic plant exsudates is high. These conditions create a specific microniche for soil microbia. Filamentous fungi typically explore larger soil volumes, and it is unlikely that species of filamentous fungi are confined to this zone. On the contrary, the mycelium of mycorrhizal fungi is generally thought to extend beyond the depletion zone, providing better access to mineral nutrients. However, if it is true that most of the mineral nutrients absorbed by plant

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roots are delivered by mycorrhizal fungi, the concept of the rhizospere and of the depletion zone needs to be revised. Fuelled by plant derived carbohydrates, actively absorbing and exsuding fungal hyphae would create their own depletion zones and microhabitat, and it may be more appropriate to use the term **mycorrhizosphere** (DUPONNOIS et al. 2008).

Roots are the primary target of many soil-born **plant pathogenic fungi**, most serious damage to crops or natural vegetation is often caused by introduced species, such as certain *Phytophtora* spp. Certain primitive plant pathogenic fungi with motile spores are known as vectors of plant viruses, demonstrating that vector properties are not limited to animals (CAMPBELL 1996).

The molecular revolution: novel fungal lineages, surprising diversity

About 20 years ago, the development of fungal specific PCR primers targeting nuclear ribosomal DNA, later combined with emerging DNA sequencing technology, opened up a whole new research area, the molecular ecology of root-fungus associations. Ever since, much of our knowledge about the identity, diversity and function of root associated fungi had to be revised.

Studies of root associated fungi continue to reveal surprising diversity, both in terms of species richness and in terms of deeply branching phylogenetic lineages. Despite significant progress in the representativity of published fungal sequence data, some of the fungal lineages discovered during early diversity studies (e.g. VANDENKOORNHUYSE et al. 2002) are still unidentified. It appears that our present knowledge about the biology of root associated fungi is based on a rather narrow selection of certain plant pathogens and of a few model species of mycorrhizal fungi. FITTER (2005) concludes that `soils contain more uncharacterized biodiversity than any of the rest of the terrestrial biosphere and it is partly that diversity that renders their behaviour (in global change) so difficult to predict´.

A case study: diversity of Sebacinales

The Sebacinales are an inconspicuous group of `primitive´ basidiomycetes, which had received little attention, before it was discovered with molecular tools that they are involved in an apparently unlimited multitude of root associations (SELOSSE et al. 2002, 2007; URBAN et al. 2003, WEISS et al. 2004, WEISS et al. 2011). Research was further stimulated by the availability of pure cultures of *Piriformospora indica*, a member of the Sebacinales.

In the past years, the ubiquity of sebacinalean root associates was recognized, but the species diversity is still far from being disentangled. According to our current knowledge, the Sebacinales are deeply split into at least two phylogenetic groups, one comprising the ECM species including certain orchid root colonizers, and another more heterogenous group involved in a multitude of plant root associations (WEISS et al. 2004, WEISS et al. 2011).

Much of sebacinalean diversity seems to be cryptic, with few morphospecies comprising several potential biological species. Currently, most ribotypes (genetically distinct biological units as defined by ribosomal DNA sequences) are known only from DNA directly amplified from roots or rhizoids of a wide diversity of plant species, including typical ECM, ERM and AM host plants, as well as orchids and liverworts. The teleomorphs (sexually reproductive structures) of these fungi are known for a few ribotypes only. A phylogenetic tree based on a BLAST generated selection of the 100 sequences most similar to a partial nuclear ribosomal large subunit DNA sequence of *Sebacina allantospora* illustrates the dominance of DNA sequence information directly genrated from plant roots, and the lack of knowledge about the fungal species (*Figure 1*). Currently field collected basidiomata of the ECM forming subgroup are analysed to fill that gap and to prepare a revision of that group. However, at present it is not certain if the limited number of available morphological characters will suffice to recognize all biological species, since phylogenetic diversity excceds by far morphological diversity. Probably, the major driver in the evolution of Sebacinales was the recognition of the diverse host species, and appartently there was only limited advantage of morphological differentiation and complexity.

A very similar situation was found in the Glomeromycota, the mycobionts of AM. This group was studied intensively, since its significance for plant growth and ecosystem services has been recognised for long. The biology of these obligately biotrophic fungi is peculiar, with coenocytic hyphae harbouring popolations of genetically diverse nuclei. No sexual state is known, rendering the application of conventional species concepts questionable. Species recognition is limited by the paucity of available micromorphological characters, about 150 two 200 species are currently recognized, but this number is probably much too low to represent the genetic diversity adequately (FITTER 2005, FITTER et al. 2011). Unresolved taxonomy resulted in confusion regarding experimental model organisms. ‘*Glomus intraradices* DAOM197198’, a model fungus in arbuscular mycorrhiza research, is not conspecific with the type culture and should be named *G. irregulare* (STOCKINGER et al. 2009).

In the Sebacinales and beyond, it appears that a significant proportion of fungal diversity is root associated. Consequently, environmental sequences, of which root associated sequences form a major part, are a complementary and increasingly important source of information about fungal biodiversity.

From biodiversity to function – the evolving molecular toolbox

There is little disagreement that mycorrhizal fungi are essential for plant productivity, element cycles, ecosystem resilience and a variety of ecosystem services. On the other hand, it is a difficult task to directly link root fungal community composition to emergent ecosystem properties, with other words, to synthesize population and community ecology and ecosystem ecology. Conceptually, the integration of different levels of organisation is at the core of ecological theory. In practice, the level of integration in ecological research and theory is limited by the potential of the methods of investigation, and by traditional borders between different disciplines of ecology (FITTER 2005).

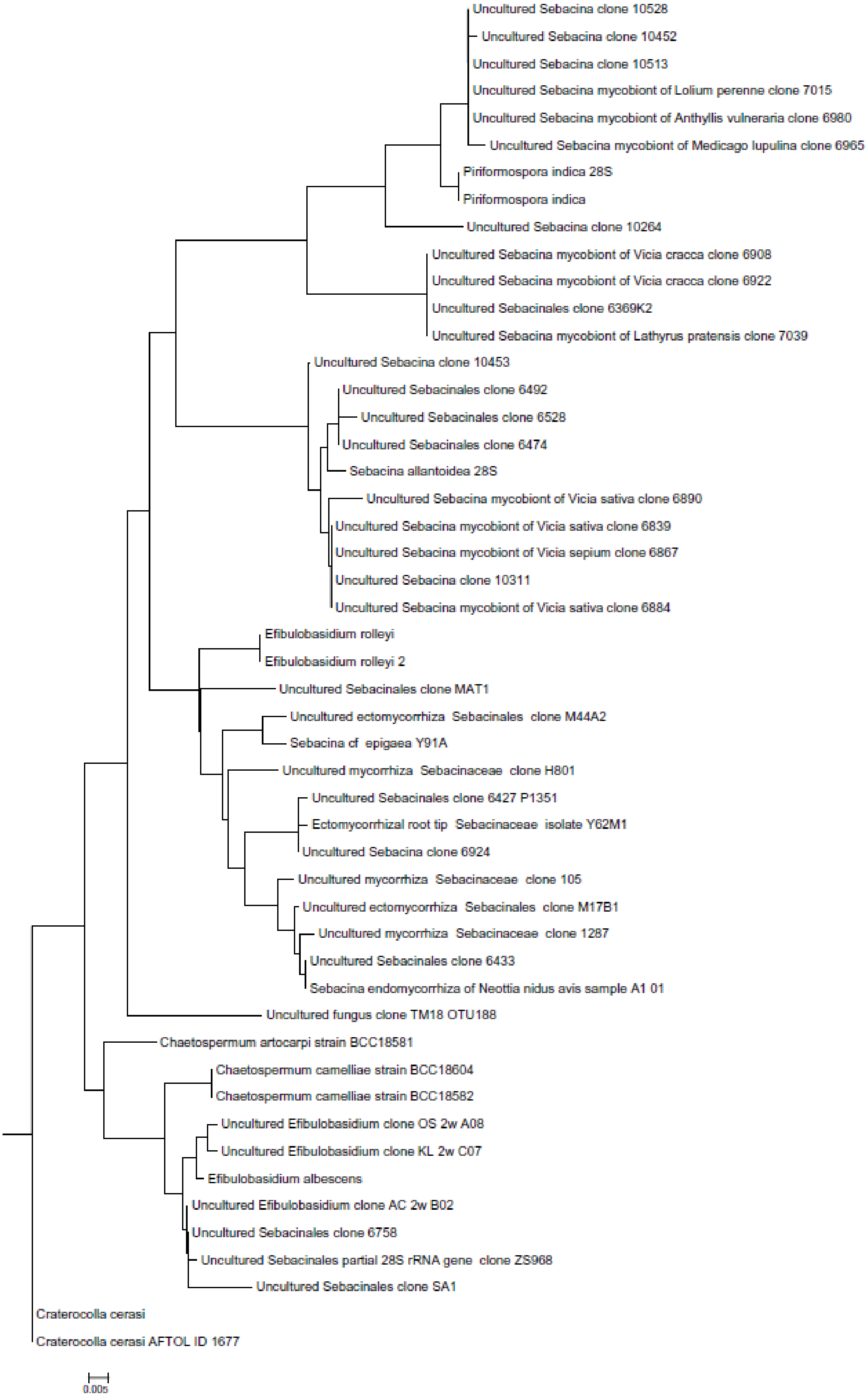

Figure 1: **Fast Minimum Evolution tree generated by the NCBI BLAST server based on BLASTN 2.2.25+ (http://blast.ncbi.nlm.nih.gov/Blast.cgi) results for a partial DNA sequence coding for the large subunit nuclear ribosomal RNA of** *Sebacina allantoidea* **(Accession number AF291367.1). Default parameters, sequence subrange basepairs 50-500, maximum target sequences = 100. Sample descriptors were automatically truncated.** *Craterocolla cerasi* **was choosen as root by the author.**

Metagenomics, metatranscriptomics and other metadisciplines promise to facilitate a unified analysis of diversity and function. The direct sequence analysis of expressed genes theoretically allows assessing both function and phylogenetic affinity simultaneously. In reality, the application of this approach is still limited by the resolution of available reference data, particularly taxonomic resolution. In an attempt to overome this limitation for one ecologically highly relevant gene, GORFER et al. (2011) established a database for fungal nitrate reductase genes, and demonstrated the applicability of the data for the identification of environmental cDNA samples. The study further suggested the multiple occerence of gene transfers of nitrate reductase encoding genes in the fungi.

With the increasing availability of bacterial and fungal genome information it will be possible to extend the metagenomic approach to a variety of fungal genes, and to assess the ecological roles of different groups of root and soil microbiota more precisely.

Applications

Up to now, the taxonomic resolution of most soil ecological studies is low. For instance, the ratio fungal biomass / bacterial biomass is regarded as an indicator of sustainability (de VRIES et al. 2006). The evolving toolbox promises to achieve a new synthesis of biodiversity research and functional ecology, and to solve fundamental theoretical questions: e.g. linking aboveground – belowground biodiversity, connecting microbial community ecology and ecosystem resilience, nutrient capture and storage efficiency, microbial community structure and composition and invasibility of plant communities, microbial biodiversity in `extreme´ environments, etc.

Are there practical applications? Purposeful management of diverse microbial communities is a relatively recent field. Mycorrhizal inoculum and gut microbes for man and farm animals are pioneer applications, with growing markets. The cultivation of mycorrhizal fungi as a crop is currently applied to narrow selection of high value species only, particularly truffles. In the past, his application has been limited by difficulties in assessing fungal identitiy and growth in the soil. New analytic tools are hence most significant for the development of this field. Other promising applications are microbiological soil pathogen control, the reduction of fertilizer input, or, more generally, the assessment of the effects of soil managent practices on microbial communities.

Conclusions

Root associated fungi are a major frontier of biodiversity research. Very basic questions are still poorly resolved, from alpha taxonomy (the naming and classification of organisms) to the quantification of biomass of fungal species and to the linking of species to processes. Deeper knowledge of the diversity, biology and ecology of root associated fungi is urgently needed to unterstand the interactions of their communities with ecosystem processes.

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