
Mycological Research News¹

Mycological Research News features: Checks and balances in endophyte-host interactions; In this issue; *TrichoBLAST*: a multilocus database for *Trichoderma* and *Hypocrea* identifications; Did pathogenic fungi contribute to dinosaur extinction?; and Forthcoming international mycological meetings.

This part starts with a major review of the nature of endophytic associations involving fungi, and includes eight other research papers. These describe a new genus associated with ambrosia beetles, characterise *Scutellospora reticulata*, analyse a chaperone homologue gene in *Paracoccidioides brasiliensis*, examine growth responses of soil chytrids, revise the hyphomycete genus *Piricauda*, assess the status and relationships of *Agaricus* mushrooms in sect. *Duploannulati*, report decreasing effectiveness of prochloraz-manganese to control *Verticillium fungicola* over time, and examine the mineral contents (including metal ions) in *Amanita* fruit bodies.

The following new scientific names are introduced: *Dryadomyces* gen. nov.; *D. amasae*, and *Piricauda vulcanensis* spp. nov.; and *Acrodictys caribensis* (syn. *P. caribensis*) comb. nov.

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CHECKS AND BALANCES IN ENDOPHYTE-HOST INTERACTIONS

The term ‘endophyte’ encompasses a very broad and largely uncharacterized diversity of microbe-plant interactions. Do fungal endophytes represent a unique fungal lifestyle, or a heterogeneous assemblage of different fungal life history traits? Mutualistic interactions are frequently emphasized as being a feature of associations between endophytic fungi and their hosts, particularly for the balansiaceous endophytes of grasses, while the numerous inconspicuous fungal infections of plants that have weakly antagonistic (pathogenic) or neutral interactions usually receive less attention. In this issue, Schulz & Boyle (pp. 661–686) provide a comprehensive review of the diversity and biology of fungi involved in asymptomatic colonisation of plants, with particular emphasis on physiological interactions between endophytes and their hosts. They propose a conceptual model for endophyte-host interactions in which symptomless occupation of plant

tissues involves a balance between fungal aggression and host defence. This balance is dynamic and can be altered by changes in physiological condition of either host or fungus, affecting potential outcomes. In reviewing the diversity of symptomless fungal colonists of plants, Schulz & Boyle cite examples of fungal endophytes illustrating a range of life-history traits and comprising a continuum of interactions from mutualism to antagonism. Fungal endophytes also display a range of host specificity from nonselective to highly host specific. Schulz & Boyle consider the continuum of interactions and variability in outcomes to be both a consequence of and a selective force in fungal speciation.

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IN THIS ISSUE

In addition to the major review on endophyte biology highlighted above, eight research papers are included in this issue. The first two combine ultrastructural and molecular techniques; one describing a new conidial

genus and species of ophiostomatoid fungi from ambrosia beetles in Tawian (pp. 687–696), and the other characterising the AM-fungus *Scutellospora reticulata* (with detailed comparative data on cell wall structure) (pp. 697–706).

The DNA encoding chaperone protein homologue in the medically important *Paracoccidioides brasiliensis* has been sequenced and compared phylogenetically with that of other fungi (pp. 707–716). This is a highly conserved gene and is in a group where protein formation is inducible under stress conditions. Studies on the

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effects of temperature on the growth of chytrids cultured from a range of Australian soils indicate that soil temperature may be a limiting factor in their distribution (pp. 717–722).

Further systematic papers include a revision of the hyphomycete genus *Piricauda* in which eight species are accepted, one being described as new (pp. 723–728), and studies on several endemic and rare *Agaricus* species (pp. 729–741). This last study, using ITS sequence data, supports the recognition of several rather local species and contributes to a re-appraisal of the sectional classification. A survey of the sensitivity

of *Verticillium fungicola*, causal agent of dry bubble disease in *Agaricus bisporus*, to prochloraz-manganese showed that the effectiveness of the fungicide decreased over seven years (pp. 742–746); mushroom growers cannot therefore rely on this compound for long-term control of the disease in their farms. Mushroom fruit bodies can concentrate elements from the soil; here the minerals in fruit bodies of eight *Amanita* species from different parts of Hungary are compared and reveal that the contents of chromium, nickel and vanadium are the most variable (pp. 747–751).

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TRICHOBLAST: A MULTILOCUS DATABASE FOR TRICHODERMA AND HYPOCREA IDENTIFICATIONS

The genus *Hypocrea* (anamorph *Trichoderma*) is of major importance as producers of industrial enzymes, biocontrol agents against phytopathogenic fungi, and as opportunistic pathogens of immunocompromised humans (Druzhinina & Kubicek 2005). Yet because of the significant homoplasy of morphological and even phenetic characters, species identification now chiefly relies on DNA sequence data, although the value of the morphology-based interactive key developed by Samuels and co-workers² should not be underestimated for those skilled in the study of such characters. When using sequence data, the most reliable results can be obtained either by using a DNA barcode (Druzhinina *et al.*, unpubl.) or from a phylogenetic analysis of several unlinked DNA fragments with known levels of intra- and interspecific variability (phylogenetic markers). However, since both of these methods are laborious, the most common approach for molecular identification is by local alignment comparison, i.e. by submitting a query sequence to a sequence repository as reference, to which it becomes aligned and from which the best matches are extracted, most popularly using BLAST (Altschul *et al.* 1997). Unfortunately, the results from large public databases like GenBank, can be erroneous as: (1) such databases are riddled with misidentified sequences (~40% for *Hypocrea/Trichoderma* in GenBank; Kopchinskiy, Kubicek & Druzhinina, unpubl.); and (2) not all public databases contain the full range of sequences of species in a genus. Consequently, it is often difficult to assess the result of BLAST when only partial or incomplete sequences are retrieved; this is of critical importance, as authors often make identifications based on the best BLAST match (lowest E-value), regardless of alignment scores.

In order to eliminate the later two difficulties with respect to *Hypocrea/Trichoderma*, we have developed *TrichoBLAST*: a publicly available database supported

by sequence diagnosis and similarity search tools, which covers all 88 genetically characterized species of the genus and contains almost complete sets of five of the most frequently used phylogenetic markers: ITS 1 and 2; two introns (*tef1_int4*(large), *tef1_int5*-short) and one exon *tef1_exon6*(large) of the gene encoding translation elongation factor 1- α ; and a portion of the exon between the 5th and 7th eukaryotic conserved amino acid motives (Liu *et al.* 1999) of subunit 2 of the RNA polymerase gene (*rpb2_exon*). *TrichoBLAST* is located on the website of the ICTF (IUBS/IUMS International Commission on the Taxonomy of Fungi)'s International Subcommittee on *Trichoderma* and *Hypocrea* (ISTH; www.isth.info).

As there is as yet no consistency amongst the *Trichoderma* community as to which primers to use for amplifying and sequencing large loci like *tef1* for identification or phylogeny, there is a considerable variation in the length and fragment area of sequences deposited in public databases under the same gene name. Consequently, the accuracy of a similarity search can be seriously corrupted: for example, if sequences of *tef1* containing both the short, highly variable intron and also a long portion of the conserved exon are submitted to BLAST, the 'best hit' will be calculated from the high score for exon-exon alignment while the intron-intron similarity will be neglected. In practice it can be exemplified by the submission of the whole *tef1* sequence of *H. jecorina* (GenBank Z23012) to either NCBI or *TrichoBLAST* both containing several records of 4th and 5th *tef1* introns of this species but none of the largest 6th exon. For reasons explained above, the genetically distant *H. melanomagna* or *H. lutea*, for which large exons are deposited, will appear as the 'best hit'. To eliminate this obstacle we have enforced *TrichoBLAST* by *TrichoMARK*, a script specifically written for the detection and retrieval of phylogenetic markers in query sequences, and for the subsequent individual submission of them to the similarity search. The first version of *TrichoMARK* is

² <http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm>

able to diagnose ITS1 and 2 sequences of *Hypocrea/Trichoderma* based on genus-specific oligonucleotide sequences both on 5' and 3' ends of the marker (Druzhinina *et al.*, unpubl.) and retrieves the exact area of the ITS1 and 2 phylogenetic marker with no flanking scraps. Further, in the case of the highly diagnostic *tef1* introns, *TrichoMARK* searches for conserved and genus specific areas framing the two introns, retrieves each intron individually for the similarity search, and also provides the results with a comparison of the actual and theoretically expected length of the detected phylogenetic marker. Finally, for the two other loci (*tef1* and *rpb2* exons, respectively), the program scans for specific oligonucleotide stretches in each gene to retrieve a fragment from the query sequence which absolutely matches that of the corresponding phylogenetic marker in *TrichoBLAST*. This approach, i.e. performing the similarity search for each phylogenetic marker individually and using a predefined area of the query sequence, significantly increases the accuracy of the method.

After this first step, the user can then automatically transmit its sequence to the similarity search, a core component of *TrichoBLAST* based on a local stand-alone BLAST server originally developed by NCBI. The result will be presented in the standard way by a combination of a graphical overview and a list of most similar sequences. However, since BLAST alignment scores represent a measure of similarity, but similarity does not represent a sound measure of relatedness (de Queiroz 1992), users should take the following factors into account when interpreting the results: (1) which phylogenetic marker was used; and (2) whether *TrichoBLAST* contains the respective sequences for all known species. As to the first point, our experience with more than 1000 vouchered ITS1 and 2 sequences representing all genetically characterized *Hypocrea/Trichoderma* species shows that ITS1 and 2 is very diagnostic and unique (Druzhinina *et al.*, unpubl.). Therefore, *TrichoBLAST* contains members of every ITS1 and 2 haplotype known from *Hypocrea/Trichoderma* today, implying that species identification is possible where there is an exact match with a sequence from the database, with the exception of five cases where two or three species share identical ITS1 and 2 haplotypes (*viz.* *T. tomentosum/T. cerinum*; *T. longipile/T. crassum*; *T. koningii/T. ovalisporum/T. muroiana*; *H. lutea/H. melanomagna*; *T. longibrachiatum/H. orientalis/H. cerebriformis*; Druzhinina *et al.*, unpubl.; all these exceptions are indicated in *TrichoBLAST* results). Cases where the ITS1 and 2 sequences do not exactly match any record in the database but differ by one or a few nucleotides are indicative of either an as yet unknown allele of a known species, or could represent a new species. To confirm the species identification in such a case, a similarity search in *TrichoBLAST* using other phylogenetic markers and/or the multilocus phylogenetic analysis will be necessary. On the other hand, for the two *tef1* introns, sequence identities are

rare due to the high level of intraspecific variability known for these markers (Druzhinina *et al.* 2004, Chaverri *et al.*, 2003). We have therefore carefully checked whether the species with the lowest E-value and highest bit score would resemble the correct identification, and were able to prove this in all.

A special case is the extremely high level of intraspecific genetic variability expressed by all known phylogenetic markers for *H. lixii/T. harzianum s. lat.* It is likely that based on the multilocus analysis by Chaverri *et al.* (2003) and our own study (Druzhinina *et al.*, unpubl.) that *H. lixii/T. harzianum* should perhaps be divided into several phylogenetic species. *TrichoBLAST* consequently considers the complexity of *H. lixii/T. harzianum* by covering the wide range of its intraspecific variability with 21 records for ITS1 and 2, and 21 and 26 records for the 4th and 5th *tef1* introns, respectively.

A difficult situation arises in the case of high similarity scores with either *tef1* or *rpb2* exons. These two markers provide a very low level of interspecific resolution for *Hypocrea/Trichoderma* (Chaverri & Samuels, 2004), and are essentially useless for species differentiation. However, as they show reliable grouping at the clade level, we recommend using these two phylogenetic markers mainly as supportive data to identifications made on the basis of *tef1* introns (preferably the *tef1_int4th-large*) and ITS1 and 2. Also, *TrichoBLAST* currently contains only a few sequences of *tef1* and *rpb2* exons from species of sect. *Longibrachiatum*, thus making these markers inappropriate for BLAST identification in this group.

Under the above caveats, matches of lesser extent are indicative of relatedness at best; however, hypotheses of relationships should not be inferred directly from similarity indices, but use phylogenetic inference methods. To this end, we have developed a third module of *TrichoBLAST*, i.e. a publicly available Multiloci Database of Phylogenetic Markers (MDPM), a relational database built on a MySQL platform running on a Mandrake Linux Apache httpd server. It is accessed through a web interface system written in PHP scripting languages. This database serves as a reference database for the similarity search and contains only records exactly corresponding to five selected phylogenetic markers, which we trimmed after the manual retrieval and alignment of published sequences. In many cases, for example for *tef1*, two or even all three fragments were extracted from the single GenBank record. MDPM allows three major ways to sort the (currently more than 600) sequences according to: (1) the phylogenetic position of the query species (*spec_key* field; Druzhinina *et al.*, unpubl.); (2) the alphabetic order of species names; and (3) phylogenetic markers (*locus* field). Moreover it allows the retrieval of any custom set of sequences in FASTA file format for phylogenetic analysis. A search option by any keyword is included to speed up the access to sequences from known strains, species, or GenBank accession numbers.

TrichoBLAST and its associated tools complements existing local databases of vouchered sequences for the identification of selected groups of fungi such as ectomycorrhizas (UNITE; <http://unite.zbi.ee>) or *Fusarium* spp. (FUSARIUM-ID v. 1.0; Geiser *et al.*, 2004). However, these examples are based on sequences from a single locus only, and also are not easily retrievable as a set ready for subsequent phylogenetic analysis. *TrichoBLAST* has the advantage of using multiple loci, enabling the detection of the correct fragment within the user's sequence for the similarity search (BLAST), and also provides direct access to sequences and their annotation. Finally, we note that *TrichoBLAST* will be continuously updated by the inclusion of sequences from more species and(or) loci and(or) haplotypes as they become available.

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DID PATHOGENIC FUNGI CONTRIBUTE TO DINOSAUR EXTINCTION?

An intriguing speculation that fungi might have contributed to the demise of the dinosaurs has just been published (Casadevall 2005) which is certain to attract wide interest. Casadevall, who is a specialist in the understanding of virulence in infectious diseases at the Albert Einstein College of Medicine in New York, starts his discourse from the observation that systemic fungal diseases are relatively rare in healthy and uncompromised mammals (including humans), compared with those caused by bacteria and viruses. He argues that the reason for this is likely to be a combination of high body temperatures and layered immune defence systems. Interestingly, systemic fungal pathogens of mammals always seem to have a natural source in the environment, usually soil, and some strategies developed by *Cryptococcus neoformans* and other fungal pathogens to avoid predation by amoeboid protozoans in soil (e.g. encapsulation) appear linked to increased virulence to mammals. At the Cretaceous-Tertiary (K-T) boundary, now dated to 300 K yr after the meteor collision that caused the Chicxulub crater in Mexico, there was widespread deforestation and a massive fungal proliferation, attributed to decreased sunlight and cooler global temperatures (Vajda & McLoughlin 2004). Casadevall considers this would have created large concentrations of fungal spores in the atmosphere that could serve as inoculum for living organisms, and suggests that the evolution of high

body temperatures (endothermy) seen in mammals could have arisen as a selection mechanism against infections by fungi in particular. Fungi are common pathogens of cold-blooded animals, so an increased biomass of infective fungal propagules could have placed many organisms at risk, not least the dinosaurs. He also notes that the only modern descendants of the dinosaurs are birds, which are warm blooded, and so would have had more resistance to fungal infections. While the hypothesis is difficult to test, there is a circumstantial case that endothermy provides increased fitness in a fungal-rich world. This is a fascinating idea that inescapably draws attention to the possible influence of fungi in shaping the Earth as we know it, in the same way as did the earlier recognition of the role of mycorrhizas in the evolution and spread of vascular plants (Malloch, Pirozynski & Raven 1980). The idea merits floating in all basic mycology courses, where it is sure to generate interest and debate.

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