Mycological Research News¹

This issue of *Mycological Research News* features: Standardized data on 474 fungal metabolites; Doubt over the identity of soma; Additional evidence for recombination in *Candida albicans*; Unexpectedly high diversity in leaf decomposition in water and Synthesys: the world's largest network of natural history institutions.

Original research papers in this part examine phosphate transfer in ectomycorrhizal fungi, phosphate efflux in arbuscular mycorrhizal (AM) fungi, and the diversity of AM fungi associated with aquatic plants.

The frequencies of dsRNA elements in *Helicobasidium mompa* and *Rosellinia necatrix* have been assessed, and that in *H. mompa* has been characterized. The separation of *H. mompa* from *H. brebissonii* is also examined.

A PCR-based detection method for *Rhizoctonia solani* on rice, and a genetic transformation system for use in *Pochonia chlamydosporia*, have been developed. Endophytic *Phoma medicaginis* can produce brefeldin A which inhibits some phylloplane fungi.

Molecular phylogenetic studies included an examination of *Mycosphaerella* species on *Eucalyptus* in South Africa, *Aphanomyces* species, ophiostomatoid fungi on pine beetles, and report a novel *Leptographium*. A new family and genus are described in the *Glomeromycota* for two species formerly referred to *Glomus*.

The following new scientific names are introduced in this part: *Gerdemanniaceae* fam. nov.; *Gerdemmania* gen. nov.; *Letographium bistatum*, *Mycosphaerella fori*, and *Ophiostoma pulvinisporum* spp. nov.; and *G. chimonobambusae* (syn. *Glomus chimonobambusae*) and *G. scintillans* (syn. *Glomus scintillans*) combs. nov.

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IN THIS ISSUE

The first three papers in this issue concern the physiology and ecology of mycorrhizas. Phosphate transfer is a key feature of ectomycorrhizal associations, and factors influencing this have been studied in cultures of three species using radioactively labelled phosphorus; the effects of phosphate availability, different cations, and also various carbohydrates are reported (pp. 599–609). By the use of inhibitors, phosphate efflux in isolated hyphae of the arbuscular mycorrhizal *Gigaspora marigata* is shown to be linked to the activity of hyphal phosphatases (pp. 610–615). Studies of the colonisation and molecular diversity of arbuscular mycorrhizal fungi of two aquatic plants in Sweden, revealed that eight phylotypes were involved and that the intensity of colonization was similar to that found in grassland (pp. 616–625).

Double-stranded RNA (dsRNA) elements in the root rot pathogens *Helicobasidium mompa* and *Rosellinia necatrix* were found at frequencies of 68.4% and 20.9% respectively, and proved removable by sexual reproduction (pp. 626–634). The dsRNA from different isolates of *H. mompa* was from about 2–<10 kbp

in size, and phylogenetic analysis suggested that one dsRNA strain was most closely allied to one known in *Fusarium poae* (pp. 635–640). Another violet root rot fungus, *H. brebissonii*, is confirmed from Japan and compared with *H. mompa* molecularly, morphologically, and pathologically (pp. 641–648).

A PCR-based method for the detection and assay of *Rhizoctonia solani* strains causing sheath blight of rice has been developed based on a long repetitive DNA sequence interspersed through the genome; sensitivity of the method is reported as an amazing 10 pg (pp. 649–653). The ability to genetically transform fungi used in biocontrol can enable visual markers and novel traits to be added. A system to do this in *Pochonia chlamydosporia* has now been developed using isolated protoplasts and plasmids carrying exemplar genes (pp. 654–661). Endophytic isolates of *Phoma medicaginis* grew rapidly on dead but not living host material; the colonized material and pure cultures contained brefeldin A which suppressed nine of 11 phylloplane fungi, suggesting the metabolite has a defence role as the fungus switches from an endophytic to a saprophytic habit (pp. 662–671).

Molecular phylogenetic studies of *Mycosphaerella* species causing leaf diseases in South African *Eucalyptus* plantations revealed that seven species were involved, one of which is newly described (pp. 672–681). Morphological and molecular comparisons

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of strains of *Aphanomyces* have been carried out for the first time, using cultures from mainly legumes in Sweden and reference strains; four species were separated, one of which was divided into two clades (pp. 682–689). The ophiostomatoid fungi associated with pine bark beetles in Mexico have been surveyed and the strains compared molecularly and morphologically; six species were recognized, of which one is described as new and two represented only by the *Sporothrix* anamorphs remain unidentified (pp. 690–698). In addition, a new *Leptographium* species with a

Sporothrix synanamorph is described from pine imported into Korea and compared molecularly with other species of the genus (pp. 699–706).

The issue concludes with a fast-tracked paper continuing a reappraisal of the glomeroid arbuscular mycorrhizal fungi. As a result of further molecular and morphological investigations, a new family *Gerdemanniaceae* is described for the new genus *Gerdemannia*, introduced here for two species formerly placed in *Glomus* but falling into a different clade which is a sister group to the *Gigasporaceae* (pp. 707–718).

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STANDARDIZED DATA ON 474 FUNGAL METABOLITES OR ‘EXTROLITE’¹

The paper of Nielsen & Smedsgaard (2003) is remarkable, and represents a pinnacle of achievement of the Danish mycology group, aided and abetted by the journal. I certainly felt awe if not shock, when I saw the work. The publishers deserve recognition for its contribution to the field, although its significance may not be fully appreciated by all mycologists.

The *Journal of Chromatography* published a TLC database for 104 secondary compounds from lichen-forming fungi in 1970 (Culberson & Kristinsson 1970). Inspired by this work, TLC data on 136 secondary metabolites from other fungi were published (Paterson 1986), and updated in Paterson & Bridge (1994). The journal subsequently produced papers compiling data using HPLC (Frisvad 1987), and then HPLC-diode array detection (Frisvad & Thrane 1987). Later was one on procedures to analyse insecticidal penicillia (Paterson & Kimmelmeier 1989), and another on UV/VIS characteristics of pure compounds (Paterson & Kimmelmeier 1990). A book on mycotoxin analysis is also available (Betina 1993). Now in this new paper, we have a treatment of 474 compounds, a major increase in the number covered compared with the 182 in Frisvad & Thrane (1987).

I remain surprised by the compound-producing capability of fungi. The number is staggering if the total number of fungi and compounds per isolates are considered. It is also instructive to be reminded how many ‘new’ compounds are being discovered. Fungi are still being used in high throughput screening programmes of some companies, despite combinatorial chemistry, although some firms have changed their strategies (Wildman 2003). The detection rate of unpublished metabolites from penicillia is still a notably high 50% (Nielsen & Smedsgaard 2003).

Of course all known fungal products are not included. For example, destruxins produced by the

biocontrol agent *Metarhizium anisopliae*. However, some of the cytochalasins from this same fungus are listed; these are highly toxic and can occur as contaminants of agricultural commodities (CAST 2003), and any increase in their occurrence requires careful regulation. The information presented can help in cases like this, although I advocate that detection limits of the methods are published in any future revision. Citation of the limits might help prevent the frequent claims that metabolites are ‘not produced’ rather than ‘not detected’. The use of toxigenic fungi which are ‘generally recognised as safe’ but which may produce harmful compounds at low levels as listed here are best avoided (*cfr* ochratoxin A from *Aspergillus niger*).

Who will use this paper? Is it predominately for drug screening purposes? Yes and no. The data will be invaluable for detecting unusual mycotoxins from fungi, total mycotoxin analysis of food/feed commodities without isolating the fungi, and permit ‘archaeological’ identifications of fungal products. While many laboratories will not have the equipment to duplicate what has been achieved here, some of the data will nevertheless have wider appeal, for example, the retention indices of compounds are of great utility. The procedures allow metabolite names to be assigned to peaks, for example in investigations into the effect of preservation on secondary metabolite production. It will also be of interest to those involved in ‘metabolomics’ (a powerful emerging technology whereby the total metabolite composition (the metabolome) of an organism is analysed (add ‘metabolomics’ to an internet search engine for more information). The paper will provide greater awareness of the potential of that approach which may no longer be eclipsed by genomics. Indeed, metabolomics should give a new lease of life to the older references quoted herein. The effects of secondary metabolites on nucleic acids can be determined more thoroughly by this method and so can provide a quality control measure for use in critical PCR work. Good reasons will now be required for *not* using these procedures in secondary metabolite studies, given the theoretical foundations and the sheer

¹ **Extrolite**: An outwardly directed chemically differentiated product of a living organism (Jens C. Frisvad, pers. comm.). This new term used by Frisvad in several lectures is preferred to ‘secondary metabolite’ as it is more explicit [Ed.].

amount of work on which this paper is based, even if access to resources means that many studies will remain further down the technological chain.

Sadly, the editing is not as excellent as are the contents, which puzzles me from such a highly regarded journal. I also wondered if some of the fungal name changes or comparisons noted were necessary, and would have appreciated a definition of the term 'dereplication'. However, do not linger on these points, but ensure there is a copy in your laboratory.

Betina, V. (1993) *Chromatography of Mycotoxins: techniques and applications*. [Journal of Chromatography Library No. 54.] Elsevier, Amsterdam.

CAST (2003) *Mycotoxins: risks in plant, animal, and human systems*. Council for Agricultural Science and Technology, Ames, IA.

Culberson, C. F. & Kristinsson, H. (1970) A standardized method for the identification of lichen products. *Journal of Chromatography* **46**: 85–93.

Frisvad, J. C. (1987) High-performance liquid chromatographic determination of profiles of mycotoxins and other secondary metabolites. *Journal of Chromatography* **392**: 333–347.

Frisvad, J. C. & Thrane, U. (1987) Standardized high performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkyl-phenone retention indices and UV-VIS spectra (diode array detection). *Journal of Chromatography* **404**: 195–214.

Nielsen, K. F. & Smedsgaard, J. (2003) Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardized liquid chromatography-UV-mass spectrometry methodology. *Journal of Chromatography A* **1002**: 111–136.

Paterson, R. R. M. (1986) Standardized one- and two-dimensional thin-layer chromatographic methods for the identification of secondary metabolites in *Penicillium* and other fungi. *Journal of Chromatography* **368**: 249–264.

Paterson, R. R. M. & Kemmelmeier, K. (1989) Gradient high performance liquid chromatography using alkylphenone retention indices of insecticidal extracts of *Penicillium* strains. *Journal of Chromatography* **483**: 153–168.

Paterson, R. R. M. & Kemmelmeier, C. (1990) UV, alkaline and difference spectra of secondary metabolites from *Penicillium* and other fungi, and comparisons to published maxima from diode array detection and gradient HPLC. *Journal of Chromatography* **511**: 195–221.

Paterson, R. R. M. and Bridge, P. D. (1994) *Biochemical Techniques for Filamentous Fungi*. [IMI Techniques Series No. 1.] CAB International, Wallingford.

Wildman, H. G. (2003) The rise and fall of natural products screening for drug discovery. *Fungal Diversity* **13**: 221–231.

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DOUBT OVER THE IDENTITY OF SOMA

Mycologists have generally been willing to accept Wasson's (1968) scholarship and arguments that soma, the divine 'plant' of immortality, was the mushroom *Amanita muscaria*. However, Wasson is now being taken to task for relying on an English translation of the *Rigveda* published 'around 1951'. Adhikari, Shrotriya & Durrieu (2003) challenge Wasson's interpretation, examining the Sanskrit texts to determine the characters of the 'plant'. The Vedic concept acknowledged only one kind of 'soma' while 24 were recognized in the Ayurveda, including *Cannabis sativa*, *Ephedra gerardiana*, and different species of *Juniperus* and *Rheum*. According to one text the original soma species became extinct in the Vedic period, and substitutes were subsequently employed. The extract is

recorded as obtained by grinding branches or piercing a tuber, and enhances strength and health but is not hallucinogenic. From the evidence presented here, it indeed seems unlikely that soma was any kind of mushroom, but determining just what it really was will require even more detective work by linguists and ethnobiologists. In the meantime, mycologists should evidently be cautious about making statements asserting that the historical soma was *Amanita muscaria*.

Adhikari, M. K., Shrotriya, J. N. & Durrieu, G. (2003) Does the plant soma exist? *Banko Janakari* **13**: 54–62.

Wasson, G. (1968) *Soma: divine mushroom of immortality*. [Ethnomycological Studies No. 1.] Harcourt Brace Jovanovich, New York.

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ADDITIONAL EVIDENCE FOR RECOMBINATION IN *CANDIDA ALBICANS*

The issue of the extent to which recombination occurs in conidial fungi and yeasts without the evident production of asci or basidia has long been a matter of uncertainty in mycology. Various methods of generating this are known, including heterokaryosis, parasexuality, hybridization, and polyploidy

(Burnett 2003). In the case of medically important fungi and plant pathogens, any recombination that occurs is of interest as it may increase virulence or limit the utility of specific detection probes. In the case of *Candida albicans*, Tavanti *et al.* (2004) used multi-locus sequence typing (MLST) for seven genes

located on four different chromosomes to investigate the haplotypes within a collection of 50 *C. albicans* strains from a variety of sources. The method revealed 46 distinct diploid strain types out of the 50 strains examined. This indicates that there is a history of clonal and other forms of reproduction in this fungus, including sexual or parasexual recombination. Indeed, it appears that homologous recombination between different haplotypes has occurred during the evolution of the strains. The results are consistent

with the hypothesis that there is a mixture of clonal and sexual or parasexual reproduction in natural populations of *C. albicans*.

Burnett, J. (2003) *Fungal Populations and Species*. Oxford University Press, Oxford.

Tavanti, A., Gow, N. A. R., Maiden, M. C. J., Odds, F. C. & Shaw, D. J. (2004) Genetic evidence for recombination in *Candida albicans* based on haplotype analysis. *Fungal Genetics and Biology* **41**: 553–562.

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UNEXPECTEDLY HIGH DIVERSITY IN LEAF DECOMPOSITION IN WATER

Traditional methods of studying fungi associated with decomposing leaves in streams favour the detection of aquatic hyphomycetes. In order to determine the spectrum of fungal groups actually present during leaf decomposition, Nikolcheva & Bärlocher (2004) used a range of primers specific for the ITS regions of different fungal groups to study tree leaves and wood which were submerged in water for four weeks in each of the four seasons. The PCR products were separated by denaturing gradient gel electrophoresis (DGGE), and the numbers of bands scored. Most fungi, as indicated by

different molecular phylotypes, were detected in the summer: these included up to 21 ascomycetes, ten basidiomycetes, four zygomycetes, 13 chytrids, and 12 straminipiles on different hosts. The data are interpreted as indicating that at least during certain seasons or particular stages of leaf decomposition, fungi other than aquatic hyphomycetes may contribute substantially to leaf decomposition in streams.

Nikolcheva, L. G. & Bärlocher, F. (2004) Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycological Progress* **3**: 41–49.

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SYNTHESYS: THE WORLD'S LARGEST NETWORK OF NATURAL HISTORY INSTITUTIONS

A consortium of Europe's most prestigious natural history institutions, including museums and botanical gardens, many of which are important for their mycological collections, have joined together to form SYNTHESYS, the world's largest natural history network. The project was initiated by The Consortium of European Taxonomic Facilities (CETAF) and links together an immense resource.

In all 20 institutions in 11 European countries are involved, which collectively hold more than 337 M natural history specimens. Those with especially important mycological collections are: The Natural History Museum, London; Royal Botanic Gardens, Kew; Royal Botanic Garden Edinburgh; Museum National d'Histoire Naturelle, Paris; Real Jardín Botánico, Madrid; Naturhistoriska Riksmuseet, Stockholm; Centraalbureau voor Schimmelcultures, Utrecht; Botanischer Garten und Botanisches Museum Berlin-Dahlem, Berlin; the Naturhistorisches Museum, Vienna; and the National Botanic Garden of Belgium, Melle.

The initiative is supported through the EU Sixth Framework Programme which aims to contribute to the creation of a true 'European Research Area',

fostering scientific excellence, competitiveness, and innovation through the promotion of better co-operation and coordination between participants at all levels. The project, which is being coordinated by The Natural History Museum, London, has been awarded £8.7 M (€13 M) from the Infrastructure Programme of the Sixth Framework.

Europe's globally important natural history collections and resources will now be available in a coordinated way to scientists across Europe as SYNTHESYS provides an opportunity for exchanging information and stimulating research. It will also facilitate access to the vast collections, and conduct a survey of each institution's collections, staff, knowledge, and expertise. The project also aims to raise scientists' awareness of best practice in handling and sampling collections through training and workshop opportunities, and the development of guidelines for the care, storage and conservation of collections.

For further information on the project and how to apply for a funded visit to one of the participating institutions, visit the project website: www.SYNTHESYS.info.