Progress Report

2004–2005

Edited by
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Centraalbureau voor Schimmelcultures
Fungal Biodiversity Centre

An Institute of the Royal Netherlands Academy of Arts and Sciences

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Preface

The CBS Fungal Biodiversity Centre, also known as the Centraalbureau voor Schimmelcultures, is one of 17 institutes of the Royal Netherlands Academy of Arts and Sciences (RNAAS). The CBS is unique in its scope and international significance, curating the world's most diverse living collection of fungi. The collection, which grows at approximately 3000 strains per year, includes organisms of crucial importance to diverse sectors of industry, as well as to agriculture and medicine. In our previous biennial report (May, 2003), we adopted the motto to **Collect, Study and Preserve**. Two years have gone by, and it is thus prudent to reflect on our vision and mission, and simultaneously assess what we have accomplished to date.

To Collect Biodiversity: In the *Studies of Mycology* volume 50, David Hawksworth provided fresh arguments for his original estimate of 1.5 million species of fungi (now accepted by many as a vast underestimate), and drew our attention to the fact that of the 7% of these species that are currently known from scientific description (approximately 100 000 species), only a subset amounting to 16% are known from culture, i.e., 1.1% of the original estimated 1.5 million species. Although the CBS collection expands more rapidly than any other, similar genetic resource centre in the world, one could ask what new projects have been initiated to further promote the collection of the "silent majority" of as yet unknown and uncultured fungi? To address this concern, the CBS, in collaboration with Agriculture and Agri-Food Canada, will officially launch the **Fungal Planet** (www.fungalplanet.org), which will aim to add a further 1% to the world's currently known fungal biodiversity, by describing 1000 new species of fungi. The concept is that descriptions of new taxa will be published and distributed on a monthly basis, and will be freely available on the web. They will also be linked via MycoBank to vouchers in herbaria, DNA banks, and culture collections world-wide. With this initiative, we hope to highlight the world's incredible fungal diversity, and to underline the importance of funding fungal biodiversity research. A major aim is to **link fungi to their environment**, i.e. the ecosystems where they occur. High quality digital colour photographs capturing the essence of each collection site as an environment are thus a prerequisite for the publication of each species description. The Big Book of Fungi, "Fungal Planet" will be compiled using material selected from the descriptions, as well as unpublished illustrations and text intended to provide a broad perspective on fungi. Our goal is to produce a book with a compelling design, as well as one full of stimulating concepts that can be used to market mycology as a serious component of biodiversity.

To Study Biodiversity: The CBS has chosen to establish various online databases via its unique BioloMICS software. A good example of such a database can be found by consulting MycoBank (www.MycoBank.org), where names of all new fungal taxa published in reputable journals will be deposited, along with the corresponding descriptions, illustrations, and voucher information (for herbarium specimens, DNA sequences and banked DNA specimens, cultures, literature citations, etc.). The CBS has chosen for a more **public engagement with science**, and is thus establishing research programmes to address issues of relevance to society. A good example of this is the inception of new postdoctoral positions for the creation of DNA barcodes to facilitate rapid recognition of fungi in various sectors such as agriculture, medicine, indoor air and food microbiology. CBS will strive for a situation where it will have a DNA sequence and barcode for each strain in the collection. This project has been initiated by means of financial support of the RNAAS, but will need considerable additional funding to attain the goal stated. As an official partner of the Consortium for the Barcode of Life (CBoL), the CBS has chosen to add DNA data to its identifications in its striving to attain a gold standard in fungal identification, and to promote a better understanding of ecological interactions where microorganisms play a role. CBS also strives to support and participate in international ventures aimed at attaining this goal, such as the US National Science Foundation (NSF)-funded *Assembling the Fungal Tree of Life* (AFToL) project.

To Preserve Biodiversity: Since our previous biennial report, the genetic resource centre has been experiencing a phase of rapid expansion. Although this is partly due to policy, it is also due to the fact that the CBS is emerging as an international collection of choice both for mycologists and for editors of high impact journals promoting the preservation of the critical voucher material and DNA extracts upon which important published identifications are based. The original mandate of CBS, when it was officially established in 1904, was based on a recommendation of the *Association Internationale des Botanistes* that an international repository must be established for fungal cultures. Soon, this mandate was broadened to include biosystematic research, and the collection and its research group were for several decades the twin pillars of CBS. In the last two years, to further strengthen the collection and international biosystematic research

on fungi, we have established MycoBank (www.MycoBank.org), the registry of new taxon names, and also have begun actively to collaborate with CABI Bioscience and Landcare New Zealand in the curation of both existing and new fungal names (www.speciesfungorum.org), linking these to unique Life Science Identifiers (LSIDs), which are supplied to GBIF. Via its MycoBank and Mycoheritage (www.cbs.knaw.nl/mycoheritage) sites CBS will be making a steadily increasing number of descriptions and illustrations available for existing names. In the coming period we will be actively developing and further improving the MycoBank software in an attempt to provide a further improved and updated service to society. CBS has also chosen for open access to scientific information. This policy gives the internet user maximal access not only to CBS databases, but also to its journal, *Studies in Mycology* (www.cbs.knaw.nl/simonline), which is now published in full colour.

In the coming two years CBS will be playing an increasingly active role in EU research programmes, striving to promote mycology and science for global public good. We will be actively expanding our culture and DNA databases, and will be establishing additional databases for specific fungal groups of interest. CBS will also take active steps to become a major training ground for young mycologists, a role that will be promoted by strengthening the interaction with top-ranking Dutch universities as well as international research bodies. In our previous report I mentioned that CBS represents a wonderful scientific opportunity as a living fungal DNA bank. Young mycologists should make it part of their education to visit the CBS. If you are within the EU, you could apply to SYNTHESYS (www.synthesys.info) for financial support to facilitate such a research visit. If you are in the U.S.A., your research professor's NSF grant will make it possible to obtain financial support for such a visit, as CBS is a member of CETAF (Consortium of European Taxonomic Facilities), which has an existing exchange with the NSF. If you are a student in a developing country, consult the web page of the Academy (www.knaw.nl), or contact us to hear about possible collaborative ventures.

Lets make the link, lets promote our science together!

Pedro W. Crous
Director,
Centraalbureau voor Schimmelcultures,
an institute of the Royal Netherlands Academy of Arts and Sciences (CBS-RNAAS)

Research collaboration

(see Programmes, Themes and Projects section for details).

Structure and Research Programmes

The CBS Fungal Biodiversity Centre is an institute of biosystematics. The primary aim of its research programmes is to enhance its unique living collection of fungi by adding valuable new data and cultures.

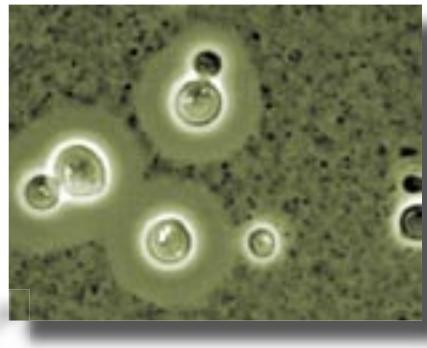
CBS has chosen to transform itself from simply being the international culture collection of choice to being the trendsetter and gold standard of mycology.

Our core business is the collection, and this is the aspect we must be the best at. Our research programmes should therefore add value to the collection. Each research programme consists of several projects. While some projects represent "discovery science", focusing on discovering biodiversity, others are focused on understanding processes, and thus on striving to unravel metabolomic, proteomic or genomic complexities of specific fungal groups or species. Additional information about these projects can be found further on in this document under the descriptions of specific research programmes.

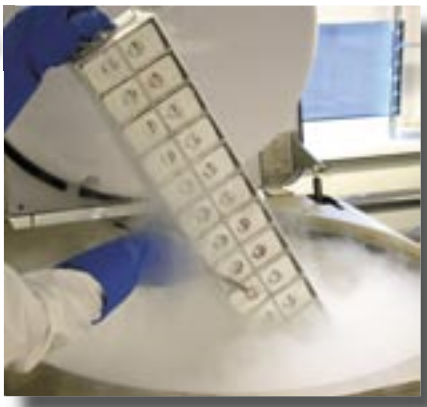
CBS is an active partner in numerous national and international collaborative projects. We aim to use these projects to broaden our scope not just in biosystematic studies but also to in the study of functional fungal biodiversity. As a partner of the Consortium for European Taxonomic Facilities (CETAF), CBS is one of the member institutes that were successful in obtaining funding from an EU project facilitating scientific exchange; the resulting program, called SYNTHESYS, subsidizes systematic researchers from the EU and EU-accession countries who want access to the collection and its facilities (www.synthesys.info). Another EU application that was awarded has funded the development of a European Distributed Institute of Taxonomy (EDIT). Within EDIT, the main task for CBS as a partner of the Netherlands Biodiversity Information Network (NL-BIF) concerns the establishment of an European network to facilitate the DNA barcoding of life. CBS also represents the Netherlands in a program developed by the Organisation for Economic Co-operation and Development (OECD), the "Biological Resource Centres" task force of the Working Party on Biotechnology. In addition, CBS as a partner within NL-BIF forms a component of the Global Biodiversity Information Network (GBIF).

Research programmes have their own themes and projects. The collection, online databases, and newly established DNA Bank serve all research programmes, and together represent the main public and scientific interface of CBS.

Evolutionary Phytopathology



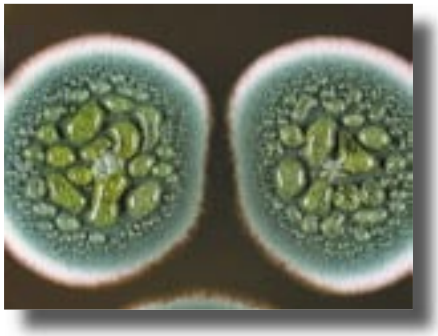
Yeast and Basidiomycete Research



The Collection

Origins of Pathogenicity in Clinical Fungi

Applied and Industrial Mycology



The Collection

The CBS Collection of Fungi has more than 50 000 strains in its public collection, making it the largest mycological culture collection in the world. CBS is unchallenged as a reference collection for mycological research, as practically all groups of the Fungal Kingdom that can be cultured are represented. In 2005, researchers from 48 countries ordered strains from our institute. CBS strains are also selected for DNA sequencing projects in the framework of global initiatives, such as the Fungal Tree of Life and DNA Barcoding. The CBS Bacterial Collection consists of another 10 000 strains, including a unique Plasmid and Phage Collection. The high quality of CBS strains is ensured by the practice of having identities and typical features authenticated by CBS specialists. Our constant endeavour to document scientific and other data with the strains increases the value of the collection to the scientific community. Much attention is given to increasing and improving our web-services, not only by digitizing publications, but also by allowing clients to use various types of collection data. CBS has developed web-based polyphasic identification for specific groups such as yeasts and Phaeoacremonium *species, and plans to extend this to include additional economically important groups. Moreover, CBS developed MycoBank, an on-line registration system for new fungal taxonomic names, as a novel service for mycology. In its most recent external peer review, the CBS Collection was rated as "excellent".*

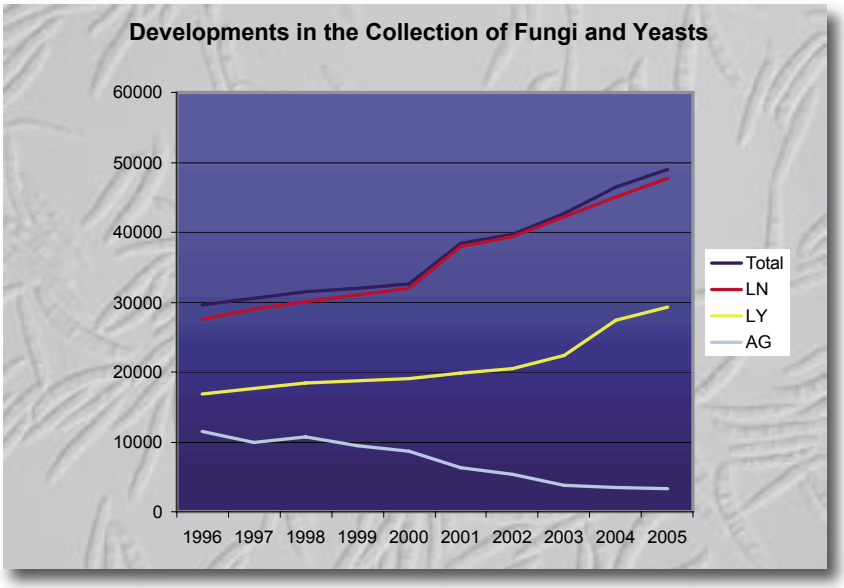
In the period of 2004–2005, CBS acquired over 5000 strains, many of them belonging to species not yet represented in the CBS collections, including many taxa entirely new to science. In the restricted collection, 38 new patent deposits were incorporated. In total, CBS now holds 944 Budapest Treaty deposits, and 263 maintenance or safe deposits. The collection of Dr K. Hyde (University of Hong Kong) which contains many type strains, was incorporated in 2004. In the past, CBS has adopted several specialized collections that had become poorly supported or orphaned elsewhere because of the retirement of specialists or because institutional policies had changed. Safe-keeping the important strains from such collections for future use is a priority for CBS.

In some cases, fungi are specifically preserved in order to rescue a threatened organism with which these fungi are associated. For example, in 2005 CBS incorporated isolates of a basidiomycetous fungus which is regarded as the natural mycobiont of *Platanthera holochila*, a nearly extinct orchid from Hawaii. The taxonomy of the fungus is still under investigation. Researchers are hoping to utilize the preserved strains in a future

recovery programme for this orchid, which is currently thought to be represented in nature by only 2–3 dozen plants. To re-establish the orchid in secure habitats, protocorms will have to be raised, and these embryonic orchids are completely dependent on successful infection with the mycobiont.

CBS preserves practically all of its strains in metabolically inactive condition, in order to ensure that the microorganisms are preserved as much as possible in the pristine original condition in which were collected or received. The most important preservation methods are cryopreservation and freeze-drying (lyophilisation). For both methods, cutting-edge equipment is available. Almost all strains are cryopreserved and stored in liquid nitrogen containers. In recent years CBS has installed gas-phase containers, in which the strains are stored in a dynamic gas phase maintained by a constant flow of cold gas from above, resulting in temperatures that are constantly below -180 °C even at the top of the containers. In conventional static phase containers this is not the case, and temperature can rise to about -120 °C, which is above the minimum temperature for potentially damaging water activity. Most yeasts and bacterial strains, and about 60 % of the non-yeast fungal strains are also preserved in a freeze-dried state. CBS uses state-of-the-art freeze-drying equipment, such as Christ-Epsilon 2-80. Some recalcitrant organisms fail to reliably remain alive after freeze-drying and cryopreservation; these strains still need to be maintained in actively growing condition on agar. However, over the past two years the number of fungal strains requiring maintenance on agar was further reduced to about 3000.

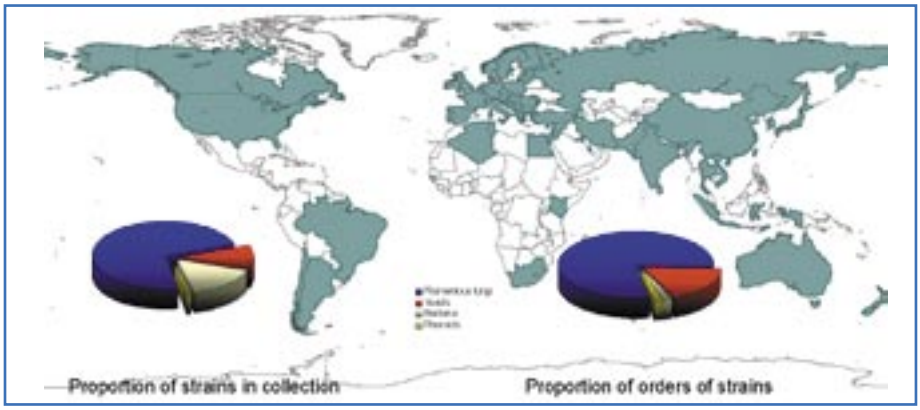
CBS uses software developed in-house to manage storage, stock control, order-handling and invoicing. The results of all control checks made on the strains over the years are recorded, allowing us to monitor the quality of each strain. Scientific information on the strains is also recorded, as is elaborated below in the section on bioinformatics and databasing.



Research projects and collaborations

DNA Barcoding of yeasts and filamentous fungi

The CBS collection currently contains over 5000 ex-type and authentic strains of yeasts and filamentous fungi. An ex-type strain fixes the undisputed application of a fungal name, while other strains that have been authenticated by a specialist also serve as reference material for the identification of a fungal taxon. These strains are becoming increasingly important now that retiring taxonomists are seldom replaced by experts on fungal identification. CBS initiated a project in 2001 aiming at sequencing selected ribosomal DNA regions (the ITS1, 5.8S, ITS2 and 26S rDNA [D1,D2] regions) of all its ex-type strains, including those now considered to be redundantly described (synonymous species names). This project will be continued in the framework of DNA Barcoding under the auspices of a partnership with the Coalition for the Barcode of Life (CBOL). Barcodes are thoroughly quality-controlled taxon-specific DNA sequences. They will be linked to the strains in the CBS database, and through BioloMICS software to MycoBank, the database for new fungal names. CBS will thus be able to provide an unprecedented service to researchers in need of rapid and correct DNA-based identifications for their isolates. Starting in 2006, postdoctoral candidates



Distribution of CBS clients.

in the new DNA Barcoding team at CBS will barcode a challenging diversity of fungal organisms. Emphasis will be given to economically important groups such as phytopathogens and medically and industrially important filamentous fungi and yeasts. Many taxa will be sequenced for the first time, and the data obtained will further phylogenetic and taxonomic research on several important fungal groups. These studies will also contribute to further Tree of Life projects.

Additionally…

• In the interlinked fields of functional genomics and bioinformatics, a project is in progress in which complete fungal genomes are being compared in order to elucidate phylogenetic and functional trends. The intention is to link evolution with the development and modification of particular metabolic and ontogenetic functions. For this project, novel software modules have been developed that allow us to obtain an improved understanding of gene expression in the organisms studied.

• The Netherlands government (Ministry of Housing, Spatial Planning and the Environment [VROM]) has made CBS responsible for routine testing aimed at detecting any accidental release of microbial contamination in laboratories working with genetically modified organisms.

• The EU MOSAICS project, which aimed at the implementation of the international Convention on Biological Diversity was concluded. In a workshop organized by the network organization European Culture Collections, the adoption of a material transfer agreement and conditions were accepted.

• Recent concern about terrorism have strongly increased the interest of politicians in culture collections. Attention is being paid to the organisms we maintain and curate, and to the way the distribution of these organisms is managed, with special focus on the security measurements instituted to prevent undesired use. For these reasons CBS participates in the OECD Working Party on Biotechnology, dealing with issues related to biosecurity (bioterrorism) in the context of the development of a global Biological Resource Centre.

• Together with G. Cardinali (DBVBAZ, Università degli Studi di Perugia, Perugia, Italy), a collaborative project was initiated to evaluate the feasibility of using the Fourier transform infrared spectroscopy (FT-IR) as diagnostic tool for yeast identification.

• In 2005, V. Robert, M.Th. Smith (both from CBS) and H.-M. Daniel (MUCL, University Catholique de Louvain, Louvain-la-Neuve, Belgium) were invited by the Life Sciences Department of Springer-Verlag to edit a two-volume handbook entitled “Yeast Taxonomy”. More than one thousand species will be described and documented in this publication that forms part of “The Yeast Handbook” series of the publisher. The two volumes are expected to be published in 2007.

• The Netherlands Culture Collection of Bacteria (NCCB), which is also housed at CBS, expanded with 39 wild-type, several acetic acid and lactic acid bacteria, and seven type strains of recently described new species. A total of 22 bacterial patent strain deposits were received according to the Budapest Treaty, and five safe deposits were accepted in the restricted collection. More than 300 bacterial strains were distributed externally.

Bioinformatics and databasing

Besides documenting its own strains and supplying an on-line catalogue, CBS is involved in several projects aiming at making the institute the web-based mycological expertise centre of the world. One of these is the Index Fungorum partnership, which makes CBS together with CABI Bioscience (U.K.) and Landcare Research (New Zealand) the custodians of the fungal nomenclatural database Index Fungorum. This database, which is freely available to the community, contains approximately 360 000 names. Each record is now assigned an LSID (Life Sciences Identifier) with the following structure: urn: lsid:indexfungorum.org:names: nnnnnn. Much effort is invested in improving the quality of the data. A list of verified pre-1832 names has been added and the "Sydow lists" (lists published by H. P. Sydow) representing all taxonomic novelties published in the years 1895–1918 have been digitized and supplemented. These lists contain about 35 000 names.

As mentioned above, CBS in 2005 launched a new initiative called MycoBank, designed to be an on-line registration system for mycological nomenclatural novelties. Authors are asked to deposit new names (new taxa and new combinations) in MycoBank, together with descriptions and illustrations. These names are checked by a programme against the nomenclatural databank to verify their uniqueness, and are checked by specialists on nomenclatural correctness as per the International Code of Botanical Nomenclature. Each name is given a unique MycoBank number that can be used in the publication, and also serves as its LSID. Collaboration with Index Fungorum prevents double issuance of LSID identifiers. Several important mycological journals have already agreed to make this procedure obligatory for their authors, while others are currently considering following suit. New software developments designed to enable mycologists to deposit data and illustrations from diverse locations worldwide are currently underway.

"NWO-groot:" large-scale support from the National Science Organisation (NWO), the Netherlands

In 2005 the digitalisation project of the four major Dutch taxonomic institutes (Naturalis, National Herbarium Netherlands [NHN], Amsterdam University Zoological Museum [ZMA] and CBS) financed by NWO started to digitize millions of herbarium specimens, and also to produce species banks for ecologically important groups of organisms. The Database Managers Committee controlling the process is chaired by CBS. The data for the 20 000 specimens present in the CBS herbarium have now been digitized. A concept for a new service referred to as "species banks" is being developed. As a pilot project, species banks are foreseen for three groups of fungi: (1) the important group of plant parasitic fungi classified in the *Mycosphaerella* complex with its anamorphs, (2) the medically important fungi included in the definitive CBS publication *Atlas of Clinical Fungi* and (3) the members of the *Aspergillus-Penicillium* complex. Species banks are planned to encompass descriptions, illustrations, sequences, a morphological data set, software to allow polyphasic identification and links to related databases and websites (e.g. PubMed, GenBank etc.).

Online publications

CBS continues to bring its publications online. Previously, this was done in collaboration with the University of Utrecht and the Royal Netherlands Academy of Sciences information institute NIWI, but in 2005 the management of CBS information was moved in-house. New volumes of the journal *Studies in Mycology* are now published simultaneously on the Web and on paper, while over 20 previously published volumes have been placed on-line. The relevant data from these books have also been transferred into the CBS descriptions database.

Mycoheritage

Bioheritage is a new initiative of SYNTHESIS to make important old works available through the Internet. CBS supports this

initiative through its new site "Mycoheritage", in which classic mycological works are displayed. A priority has been given to works containing illustrations that give insight into the historical taxonomic concepts devised by the great-grandfathers of Mycology - for example Persoon, Fries and Saccardo. The online publication of the first volume of Sowerby's "Coloured Figures of English Fungi" (1797) http://www.cbs.knaw.nl/mycoheritage/ highlights this new CBS concept. Additional books are currently being data-entered.

"Het Nederlands soortenregister" - a list of species occurring in the Netherlands - is an initiative of the Ministry of Agriculture, Nature Conservation and Fishery (familiar Dutch acronym LNV), which requested that CBS, in collaboration with the Dutch Mycological Society, provide the names of the fungi and oomycetes known to occur in the Netherlands. For the microfungi such a list had to be developed from scratch. An initial version was produced in 2005 in collaboration with the national Plant Quarantine Service (PD) in Wageningen.

The CBS databases are definitely highly appreciated. A user analysis has indicated that the numbers of visitors who actually perform a search (thus not merely the number of hits, which average 10 000 per day!) in the Index Fungorum is about 30.000 per month. The Aphyllophorales database processes about 1000 search requests per month. The yeast database, which provides both information and also an interactive, polyphasic identification tool (via BioloMICS software), is regularly used by more than 7000 researchers from 96 countries (see figure below). A new collaboration with a goal of creating several databases related to fungal human pathogens was initiated in 2005 with W. Meyer (University of Sydney, Westmead Hospital, Sydney, Australia) and will be pursued until 2007. The goal of this project (Australian grant #352303; Title: "Phylogeny as a basis for molecular identification of pathogenic fungi") is to allow Internet users to perform online polyphasic identifications that include morphological, physiological, electrophoretic and sequence data.

DNA Bank (NL-Bank)

Members of the Taxonomic Facilities in the Netherlands (NL-TAF) network are in need of a central DNA bank to store the DNA extracted during current and future phylogenetic and DNA barcoding research projects. DNA extraction from specimens and cultures is time-consuming and costly, while curation of the samples so obtained often has a very low priority in day-to-day practice of the molecular biological labs. However, when extracts are properly and centrally stored, much unnecessary repetition can be avoided. NL-TAF members have agreed to store their samples in a central DNA bank, NL-Bank, at CBS, which is the member institute that has the best developed facilities and expertise related both to handling the databases involved, and to shipping biological material, including material derived from pathogenic organisms, according to the very stringent international regulations that govern such procedures. The Royal Netherlands Academy of Sciences has provided start-up funding, and additional funding is currently being actively sought. NL-Bank will also provide a service for researchers who prefer genomic DNA extracts to biological specimens, living cultures, or tissue samples. In addition, NL-Bank will facilitate storage of DNA of extremely rare or endangered taxa. Therefore, in cases where living species or genotypes become extinct, or in cases where extremely rare or ancient specimens yield useable DNA, ongoing research can be performed based on use of the stored DNA.

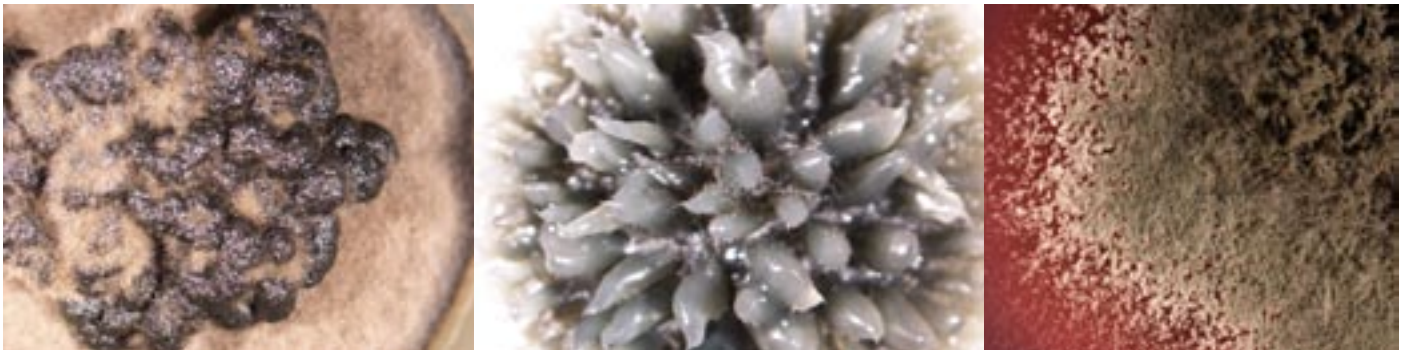
Evolutionairy Phytopathology

The importance of producing food sufficient in quality and quantity remains paramount for sustaining quality of life. Inadvertent introductions of phytopathogenic fungi have had dire consequences to nature and to cultivated crops on various continents in the past. The economic impact of such introductions can be seen in yield loss and in increased input costs for cultivation and disease control, as well as in social impact. To combat these diseases on an international scale, it is important to clarify whether the same species and genotypes occur in various countries, since each different species and genotype can be expected to have different patterns of attack, as well as different responses to fungicides and to climatological conditions. With such pathogens, it is also important to know what their host ranges and mating strategies are, and how this relates to different disease control mechanisms. The global movement of agricultural and forestry produce is inextricably cross-linked, and will continue to be so in future. Knowing which pathogens occur where and on what crops facilitates trade in agricultural produce. In this programme, we address these economically vital matters by investigating the speciation and host adaptation of various important phytopathogenic fungi.

Host specificity and speciation in *Mycosphaerella*

Thousands of ascomycetous fungal species are included in the genus *Mycosphaerella*, which has been linked to diseases on most genera of plants. Most species have been described on the assumption that they are highly host-specific, and that different plant hosts harbour different fungal species. With the implementation of molecular phylogeny as the basis of modern taxonomy, host relationships and specificity can now be tested. A major aim of our research is to determine how exclusive the host-pathogen relationship of *Mycosphaerella* species is. Investigations based on genomic analysis are in progress on fungal species from a wide range of plant hosts. *Mycosphaerella* has been linked to numerous asexual reproductive states that may have evolved into exclusively asexual species. Such asexual forms were often difficult to trace to a sexual ancestor and were thus historically placed in separate genera. One such example is the genus *Cercospora*, which represents several thousand names, of which roughly a thousand can be recognised based on morphology. A more difficult problem concerns the celery pathogen, *Cercospora apii*, which has close to 300 morphologically indistinguishable synonyms, collectively referred to as the *C. apii sensu lato* (= *C. apii* “in the broad sense”) species complex. A multi-gene sequence comparison of close to 100 species in this complex has revealed, however, that *C. apii sensu lato* consists of several functional species that are morphologically indistinguishable from one another. *Cercospora beticola*, an important pathogen of sugar beet in Europe, is one such example, and is used as model to study variation and speciation within *Cercospora*. The genus *Cercospora* appears to be largely asexual: very few species have been reported to have *Mycosphaerella* states. To investigate this matter further, mating type probes were developed to screen populations of *C. beticola*, *C. apii*, *C. zeae-maydis* (on maize), and two newly described species, *C. apiicola* (on celery), and *C. zeina*

Cercospora *leaf spot of sugar beet.*



Mycosphaerella *species in culture.*

(on maize). The results of this screening indicate that some species are undergoing cryptic sex, and probably have functional *Mycosphaerella* teleomorphs that have yet to be found, while others appear to be truly asexual.

A similar DNA phylogeny approach has also been used to investigate the evolution and inter-relationships of *Mycosphaerella* species causing defoliation and deformation of various hosts. These include species occurring on *Pinus, Eucalyptus, Acacia* (cultivated for timber, paper and pulp industries), *Olea* (olives), *Protea* (cut-flowers), and *Musa* (eating and cooking bananas). Numerous species of *Mycosphaerella* were found to be associated with the Sigatoka disease complex of banana. Several of these species appear to be confined to certain regions, while others were more global in distribution. The possibility of interaction and hybridization among these species is being investigated. Specific TaqMan probes have been developed in collaboration with Plant Research International (Wageningen University), which will facilitate the early detection and monitoring of the disease. Also, the mating type genes of *M. fijiensis*, *M. musicola* and *M. eumusae* have been cloned, and their distribution within populations is being determined. This is being done to assess the occurrence of sexual reproduction, a factor controlling genetic recombination and genotypic diversity.

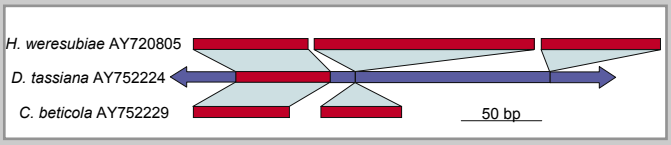
To aid our understanding of the pathology of the genus *Mycosphaerella*, two model species of *Mycosphaerella*, *M. graminicola* and *M. fijiensis*, were selected by the *International Mycosphaerella Genomics Consortium*, in which CBS participates, for complete genome sequencing. These species were selected on the basis of their economic significance to the wheat and banana/plantain industries. A joint project between the USDA-ARS/Purdue University and Plant Research International B.V. was initiated to sequence both genomes, along with 40,000 ESTs from each of *M. fijiensis* and the related maize pathogen *Cercospora zeae-maydis*. The work was conducted through the Community Sequencing Program sponsored by the U.S. DOE-Joint Genome Institute. A community-wide effort for annotation, culminating in an annotation jamboree (during 2006) will be open to all interested participants. This project will be coordinated with sequencing efforts planned for other *Mycosphaerella* species and relatives to greatly increase the power of future comparative genomics analyses.

Botryosphaeria canker pathogens

Botryosphaeria is a species-rich genus with a cosmopolitan distribution, commonly associated with dieback and cankers of woody plants. As many as 18 anamorph genera have been associated with *Botryosphaeria*, most of which have been reduced to synonymy under *Diplodia* (conidia mostly ovoid, pigmented, thick-walled), or *Fusicoccum*

Organisation of genes commonly used for phylogenetic analyses

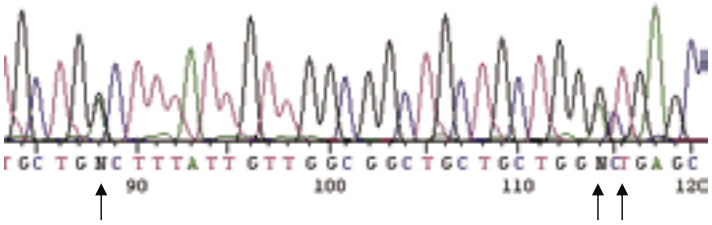
Housekeeping genes such as translation elongation factor 1-alpha, beta-tubulin, actin, calmodulin and histone H3 are commonly used as sources of nucleotide sequence data for species comparisons using phylogenetic software. Other uses of these genes include bar coding and serving as templates for species-specific primers for identification purposes. Although universal primers are available for the (partial) amplification of these genes, these primers are often designed for genera unrelated to your favorite genus, resulting in amplification failure. A second problem often encountered is that the part of a gene that is very polymorphic in one genus is not as informative in another genus (see figure below). We are currently in the process of designing new primer sets that will allow us to amplify all parts of the genes listed above. Having the complete sequences of these genes for a number of representative species from different genera available to us will allow us to identify which part(s) of the gene is more informative and therefore more useful for phylogeny, bar coding and species-specific amplification. We will also be able to track the evolution of the organisation of these genes, e.g. the position, size and number of introns, through a higher order phylogeny of a given family.



Organisation of partial calmodulin gene. The non-coding regions (introns) are indicated in red and the coding regions (exons) in blue. For this part of the gene, *Davidiella tassiana* only contains one intron whereas *Harknessia weresubiae* has three and *Cercospora beticola* has two introns. Although the first intron is approximately the same size in all three species, the second one varies greatly in size (drawn to scale) and the third is only present in *H. weresubiae*.

(conidia mostly fusoid, hyaline, thin-walled). However, there are numerous conidial anamorphs with morphological characteristics intermediate between *Diplodia* and *Fusicoccum*, and there are also several records of species outside the *Botryosphaeriaceae* that have anamorphs apparently typical of *Botryosphaeria sensu stricto.* Recent molecular studies have also linked *Botryosphaeria* to species with pigmented, septate ascospores and anamorphs in *Dothiorella*, or to species with hyaline ascospores and *Fusicoccum* anamorphs linked to *Dichomera* synanamorphs. By employing DNA sequence data for various loci, different lineages, representing 12 clades, could be resolved within the morphological concept of the *Botryosphaeriaceae*. Two of these lineages clustered outside the molecularly reconceived *Botryosphaeriaceae sensu stricto*; both were groups with *Diplodia*-like anamorphs occurring on maize. These phylogenetically disparate lineages are best accommodated in *Stenocarpella* (*Diaporthales*) and in an unresolved clade including species of *Camarosporium*/*Microdiplodia*. The ten lineages retained within the *Botryosphaeriaceae sensu stricto* represented different anamorph-teleomorph combinations, many of which are new to science. Further studies are underway to resolve the taxonomic status of many of these generic and species complexes occurring on different woody hosts.

Ascus of Botryosphaeriaceae.



Electropherogram of part of the internal transcribed spacer sequence of the ribosomal RNA gene repeat (ITS) of *Phytophthora* hybrid strains showing double bases at three positions (see arrows), where the sequences of *P. hedraiandra* and *P. cactorum* differ.

Hybridisation in *Phytophthora* and *Pythium*

Pythium and *Phytophthora* are two highly economically significant genera of fungus-like Oomycetes responsible for many types of crop disease and tree decline. The best known of the crop diseases is potato late blight (*Phytophthora infestans*), the cause of the Irish potato famine and a major agent of crop damage to this day. A study of the organization of the 5S rRNA gene family was performed for 87 species and varieties of *Pythium.* For the four different patterns of 5S organization that were found to occur within the genus, studies were conducted to determine how they arose and how evolutionarily stable they were. A number of *Phytophthora* strains were also included in the study as a reference outgroup giving insight into the ancestral organisation of the 5S gene family. The most parsimonious interpretation of the data would be that a contiguously linked arrangement of 5S sequences was the ancestral condition.

A DNA array was developed as tool for the rapid identification and detection of *Pythium* species in pure culture, as well as in environmentally mixed samples. Oligonucleotides complementary to specific diagnostic regions of ribosomal internal transcribed spacers (ITS) were designed for more than 100 *Pythium* species and varieties as well as for groups of related species. Specificity was tested in hybridisation experiments with DNA from ex-type strains and other representative strains. BLAST analyses against *Pythium* DNA sequences available in GenBank were used to confirm that species-specific oligonucleotides were unique to all the available strains of each species. In a blind test with 50 additional unidentified *Pythium* isolates from soil, the array hybridization patterns obtained were found to concur with isolate identifications obtained via morphological study and ITS sequences. In another blind test, total DNA of soil samples was amplified and hybridised on the array. Results were compared to the results of isolation by soil dilution plating and root baiting. Thirteen species were detected by the DNA array. These species corresponded with those obtained by isolation, though isolation also revealed the presence of one species that was not represented on the array. From these results it can be concluded that the DNA array is a reliable tool for identification and detection of the majority of *Pythium* species in environmental samples. Simultaneous detection and identification of multiple species of soil-borne pathogens such as *Pythium* will be a major step forward for epidemiological and ecological studies.

Investigations of a number of atypical *Phytophthora* isolates initially identified as *P. cactorum* disclosed that these isolates were actually inter-species hybrids. Isozyme analysis demonstrated

the presence of two alleles rather than the usual single allele for the dimeric malic enzyme (MDHP) in these isolates. One allele of the pair was typical for *P. cactorum* while the other was typical for *P. hedraiandra*. Sequencing of ribosomal ITS loci showed that this marker was heterogeneous in the atypical isolates, and that the sequences of *P. cactorum* and *P. hedraiandra* were both present.

Phytophthora is diploid, and hybrids are expected to combine the genetic characters of both parents as is normally seen in plants and animals (but not most fungi). The mitochondrial genome, however, is inherited maternally and will be present in a single type derived from one of the parents. Indeed, the presumed hybrids were found to possess only one type of the mitochondrial CoxI gene, either that of the *P. cactorum* or that of *P. hedraiandra*.

Two isolates showed deviating combinations of the characters mentioned above, suggesting that evolution by genome rearrangement had already taken place in some later-generation progeny of the hybrid lineages.

The hybrid *Phytophthora* isolates were found on a variety of plant hosts in public parks all over the Netherlands, making it appear highly likely that they have also become established in natural ecosystems.

Phytophthora hedraiandra is a species that has probably only recently been imported into the Netherlands via the use of Mediterranean *Viburnum* shrubs in gardening, while *P. cactorum* is a long-established native phytopathogen. The recent proliferation of hybrids between these species appears to fulfil a long-standing prediction that novel pathogenic Oomycetes would arise as world trade in plant products brought Oomycetes from around the world into interaction with one another. Several of the hosts infected by the hybrids are not known to be infected by either parent species. This suggests that such hybridizations arising from a breakdown in geographic barriers could cause the emergence of novel and unpredictable phytopathogen epidemiologies.

Petri disease and phaeohyphomycosis caused by species of *Phaeoacremonium*

Species of *Phaeoacremonium* are involved in Petri disease and esca of grapevines. Additionally, several species of *Phaeoacremonium* also cause phaeohyphomycosis in humans. During this study, *Togninia* (*Calosphaeriales*) was confirmed as teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility, and DNA phylogeny. Three species of *Phaeoacremonium* have been associated with phaeohyphomycosis. These are *Pm. parasiticum*, *Pm. inflatipes* and *Pm. rubrigenum*. Numerous unknown isolates resembling *Phaeoacremonium* spp. have in recent years been isolated from human patients, as well as from woody plants that appear to be the main environmental source of these fungi. New species were identified based on their cultural and morphological characters, and phylogenetic analyses of partial sequences of the actin, β-tubulin and calmodulin genes. A multiple-entry electronic key based on morphological, cultural and β-tubulin sequence data was developed to facilitate routine species identification.

The genus *Togninia* was monographed along with its *Phaeoacremonium* anamorphs. Ten species of *Togninia* and 22 species of *Phaeoacremonium* were recognised. Species were identified based on their cultural and morphological characters, supported by DNA data derived from partial sequences of the actin and β-tubulin genes. Phylogenies of the SSU and LSU rRNA genes were used to determine whether *Togninia* had more affinity with the *Calosphaeriales* or the *Diaporthales*. A rapid molecular identification method was developed for the 22 species of *Phaeacremonium*. It involved the use of 23 species-specific primers, including 20 primers targeting the β-tubulin gene and three targeting the actin gene. Furthermore, the multiple-entry electronic key was updated to include the new species of *Phaeoacremonium*. Separate dichotomous keys were provided for the identification of the *Togninia* and *Phaeoacremonium* species, and their mating strategies elucidated. Keys for the identification of *Phaeoacremonium*-like fungi and the genera related to *Togninia* were also provided.

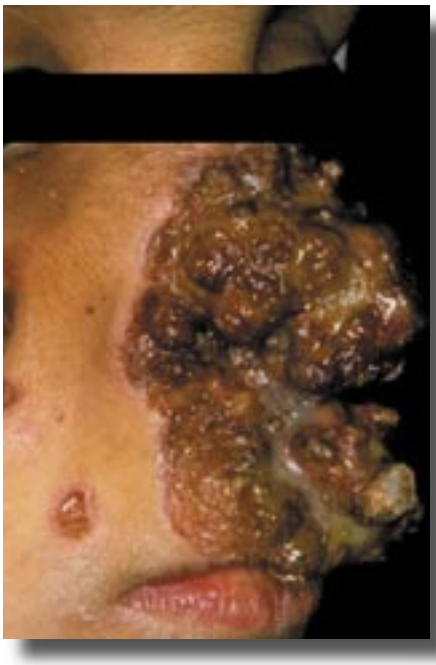
Phaeoacremonium *inoculations on grapevines.*

Origin of Pathogenicity in Clinical Fungi

Many fungi are able to cause severely mutilating and even fatal infections in humans with impaired immune systems. Remarkably, most of these "opportunistic" fungi are otherwise commonly found as harmless saprobes in the environment. How is it possible that such harmless moulds suddenly change into potential killers? And should there be a concern, parallel to our fears about bird influenza, that evolution will soon give rise to better adapted genotypes with even higher virulence? Answers to these questions may lie in the natural habitat of the fungi involved. By understanding the ecology of opportunistic fungi and the ability of these fungi to change under the pressure of changing conditions, we can begin to intelligently evaluate both the short- and the long-term risks involved.

Humans as a microbial supermarket?

We recently put forward new hypotheses concerning how fungi, after jumping from their natural niches to the human-dominated environment, eventually become potent opportunistic agents of human disease. These hypotheses are quite different from most currently held theories in medical mycology.



A patient with an infection by Phialophora verrucosa.

The most generally held idea – and from our point of view the least probable one – is that these fungi simply have a broad tolerance of adverse conditions, and thus are able to survive when accidentally inoculated into humans. A popular catch-phrase used to advocate this idea is reference to a severely immunocompromised patient, vulnerable to being infected by a wide variety of household fungi, as a "living petri dish", i.e., a supermarket for microbes.

We believe, however, that opportunism is a very rare phenomenon in the fungal Kingdom, and thus must be based on highly specific abilities. Analyzing the list of fungi from really extreme environments, we notice that extremotolerance and human infection are practically mutually exclusive.

Our present research particularly focuses on an alternative manner in which pathogenicity can develop: fungi becoming adapted to niches in the human-made environment, and, using these niches as evolutionary stepping stones, developing properties that fortuitously predispose them to be able to cause human infection. A striking example is the black yeast *Exophiala dermatitidis*, which is ubiquitous in the steam baths and hot tubs of public bathing facilities, including Asian-type public baths. This species is also known from pulmonary and cerebral infections in humans. We found its natural habitat: after a long search in a diversity of environments, we found that the intestinal tracts of fruit-eating birds and bats in the tropical rain forest were consistently positive. During the transition in which this fungus moved from its natural habitat, via the intermediary steam bath habitat to the causation of deep infection in humans, a remarkable positive selection of a single genotype took place.

Isolation work in search for the natural reservoir of a black yeast.

Hydrocarbon pollution promotes fungal infection

This suggests that anthropogenic stepping stones can facilitate evolution towards increased virulence in opportunistic fungi.

Candidates for similar evolutionary histories might be found among the brain-infecting, emerging opportunists *Pseudallescheria* and *Cladophialophora*. The natural niche of these fungi is still unknown, but they are found relatively frequently in environments polluted with agricultural manure or with toxic aromatic compounds and other xenobiotics. There appears to be an association between hydrocarbon assimilation and the ability of fungi to cause opportunistic disease in humans.

A practical consequence of this finding is that biofiltration and bioremediation techniques based on use of fungi to break down toxic wastes should be carried out in a way that minimizes risks to human health. In particular, respiratory or cutaneous exposure to specialized hydrocarbon-degrading fungi or to biofilters containing mixtures of such organisms is not recommended

Life on Mars

To critically assess the hypothesis that medically important fungi are in some way connected with extremotolerance, we conducted in-depth studies into a number of remarkably extremotolerant fungi isolated from harsh environments. These fungi, for the major part, were unknown to science and still have to be described as new genera and species.

As an example, we obtained rock-colonizing fungi in Antarctic ice-free deserts. Such fungi grow at the outermost edge of the conditions potentially supporting life, surviving average temperatures of -40 °C, and growing almost without water and nutrients while being subjected to high levels of UV radiation. Their growth rate is extremely slow, and they grow only as very small microcolonies. Such organisms could well be studied as models for extra-terrestrial life and are therefore used in modelling the possible forms of life on other planets, such as Mars. Other microcolonial fungi are known to degrade sun-exposed monuments and natural rock in the Mediterranean, growing under extreme conditions of drought and at temperatures up to +60 °C. One common factor that characterises all these microcolonial fungi, which are very diverse in terms of phylogenetic or evolutionary origin, is the development of clump-like “meristematic” growth.

In this type of growth, as seen in extremotolerant fungi, individual cells possess thick cells walls that are heavy melanised, that is, heavily shielded from solar radiation by melanin pigments that are chemically and functionally similar to dark human skin pigments. Despite these striking adaptations to adverse conditions, microcolonial rock fungi concerned have never been encountered in human infections. Fungal pathogenicity is thus more complicated than simple survival of the adverse conditions that occur in living mammalian tissue.

Moulds in our drinking water

Another project concerns the quality of municipal drinking water derived from groundwater, which is known to contain several black yeasts and the filamentous fungus *Cadophora malorum* in abundance. *C. malorum* is a member of the order *Leotiales*, which generally appears to lack human-pathogenic potential. We are beginning to understand the natural life cycle of these fungi by the discovery of identical sequences of *Cadophora* and some known teleomorph species. Our hypothesis is that these fungi are endophytes in living plants and sporulate with sexual fruit bodies on the plant after its death. Asexual conidia are dispersed through water currents.

The *Exophiala* species from cold waters, including drinking water, are known to infect cold-blooded animals such as fish. Their pathogenicity seems to be determined by their preferred temperature of growth. Species that grow easily at temperatures above 36 °C are frequently encountered in human infection;

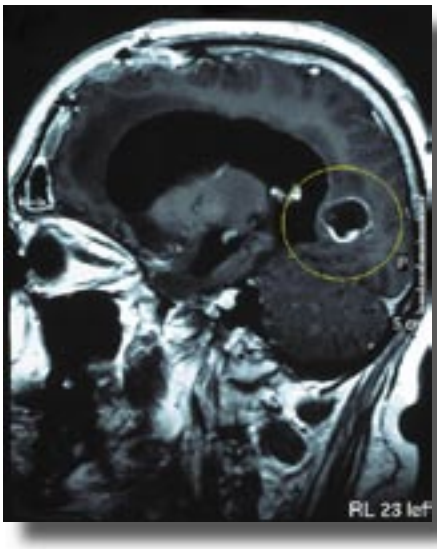
if the optimum is around 30 °C the fungi found in shallow subtropical marshes and cause infections in crabs and similar animals, while those with optima around 22 °C are opportunists on ocean fish.

Fungi with no private lives: how to evolve when sex is impossible

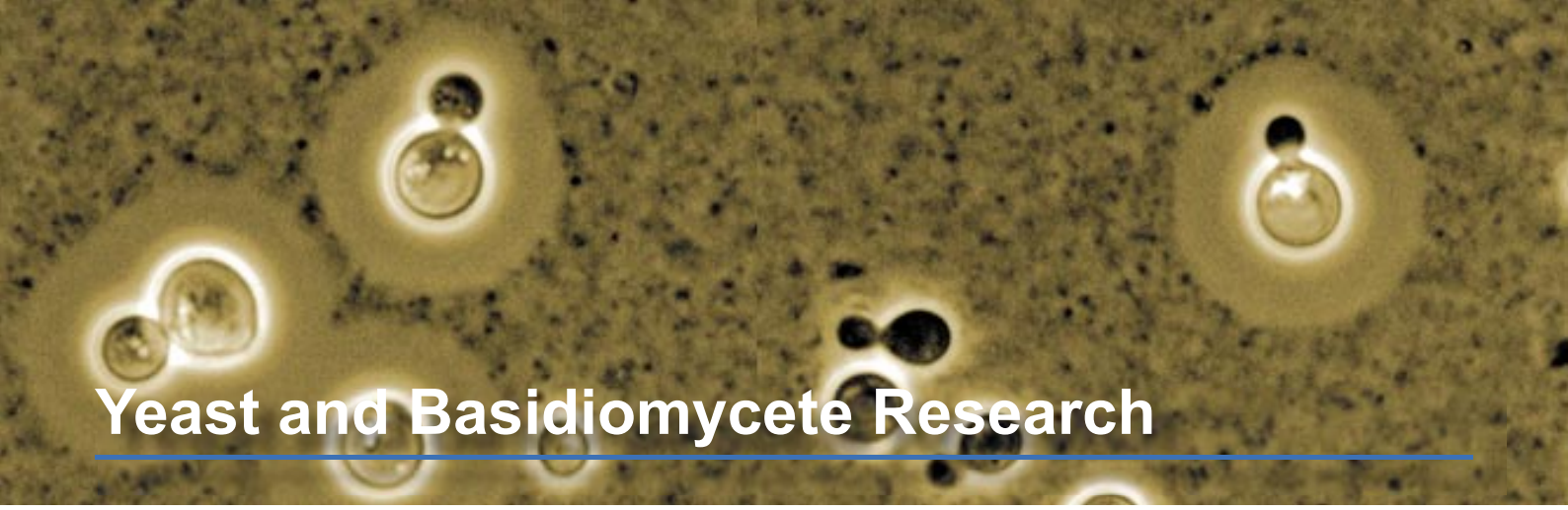
Biologists who study higher organisms explain the near-ubiquitous occurrence of sexuality in terms of the advantages of making new genetic combinations (confusing parasites, bringing advantageous genes into combination) and overcoming the genetic staleness that occurs when organisms reproduce without sex (persistence of outmoded gene combinations, steady accumulation of harmful mutations). Lack of sexuality is often thought to predispose species to rapid extinction. However, no fungus that has adapted to cause human contagious disease or commensalism (the ability to live harmlessly on human body surfaces) has ever managed to hang on to the normal sexual cycle of its ancestral fungal group. The problem is especially acute with our most common fungal pathogens, the dermatophytes (ringworm and athlete's foot fungi, a group associated more with giggles than fear, except among medical economists who know about the billions of euros/dollars per year spent on diagnosis and treatment). These fungi cannot have sex at all: if they did, they would form a little fruiting body resembling a ball of fluff on a woollen sweater, and this would immediately be scratched off by the human involved. Paradoxically, though, dermatophytes are among the most successful and adaptable human pathogens, evading modern medicine and infecting more than 70 % of humans at some point in their lives. How do they manage it? One clue seems to lie in their mating type genes (roughly equivalent to our human X and Y chromosomes). Many dermatophyte species infecting humans are suspected, on the basis of very laborious classical genetic studies, to be either 100 % "male" (minus mating type) or 100 % "female" (plus mating type). Even in sexual dermatophyte species infecting animals, there appears to be a strong "bias" towards one sex or the other in strains causing disease. Why would one sex have different pathogenic properties or better evolutionary persistence than the other? To approach this question, we are using modern molecular genetics techniques to study the mating type genes of dermatophytes, and to rapidly determine using large sample sizes what the situations are where one fungal sex has become much more successful than the other, and perhaps even become the only sex existing in the species. Genomic comparisons can then be used to catalogue what differences exist between closely related pathogens differing both in mating type and in the types of disease they cause.

"A peanut gave me this earache:" can human fungal diseases be caught from plants?

When we think of human disease, certain common sources come to mind: coughing schoolchildren, bad water, leftover food, tropical mosquitoes and so on. Plants are not high on the list of items to be feared. Yet in recent years, refined molecular genetic studies have confirmed that some plant pathogens and endophytes (fungi that grow harmlessly in healthy plants) are competent human opportunistic pathogens. For example, one of the most common causes of fungal skin cyst, *Phaeoacremonium parasiticum*, is common in grapevines, especially those diagnosed as having "esca" or "black goo" disease. Also, one of the common rots of melons and other vegetable crops related to cucumbers, "*Fusarium solani* MP V" ("mating population 5"), is one of the most common genotypes causing aggressive infections in immunocompromised bone marrow transplant patients. Conversely, a well-known fungal skin disease occurring in arid climates, chromoblastomycosis, appears to be caused by a fungus which has its natural niche in cactus spines. We are currently investigating the extent to which selected phytopathogen groups may yield competent human pathogens. Detailed molecular genetic studies are being used to be certain that any true phytopathogens involved in human disease can be distinguished from related saprobes, some of which are also agents of human opportunistic disease. Such studies could facilitate later genomic work contrasting what genes fungal isolates turn on when they are growing in animals, growing in plants, and growing on artificial media.



Brain lesion caused by Pseudallescheria boydii *after near-drowning in polluted water.*



Yeast and Basidiomycete Research

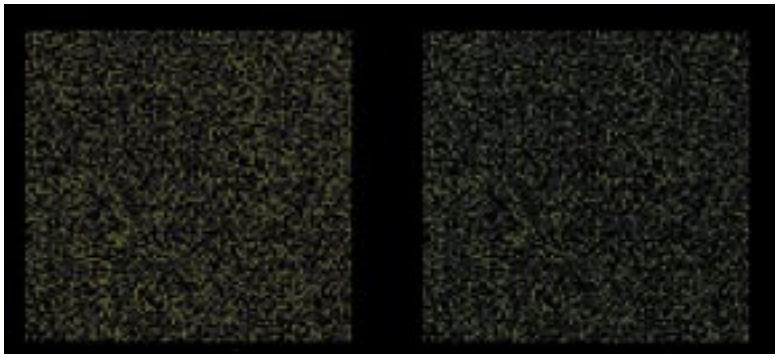
Fungi are closely related to animals, making them excellent model organisms for basic cell biological and developmental studies that are directly relevant to human biology. They have therefore become one of the most intensively studied eukaryotic groups in the rapidly expanding field of genomics, and the number of complete genome sequences available for fungal species is rapidly increasing. This unprecedented quantity of information will make an unparalleled contribution to our understanding of fungal phylogeny and evolution, as well as to our understanding of how fungal cells, and by extension all living cells, function. In this research programme we develop and explore fungal genomic data from a perspective of understanding biodiversity. This includes both comparisons between organisms and analyses of cellular functions within individual organisms. We strive to increase scientific understanding of fungal macro-evolution (*evolution of large, distinct groups of organisms*)*, speciation and inter-species hybridization events. We also assess virulence attributes, that is, properties conferring the ability to cause human disease on certain fungi.*

Human pathogenic yeasts

These research projects aim to understand the biodiversity as well as the virulence properties of selected clinically important yeast species, namely *Cryptococcus neoformans*, *Cryptococcus gattii,* and *Malassezia, Trichosporon* and *Candida* species.

***Cryptococcus neoformans*:** *Cryptococcus neoformans* and *C. gattii,* are yeasts that may cause meningoencephalitis. *C. neoformans* commonly causes severe disease in HIV-positive patients, whereas *C. gattii* infects mainly immunocompetent people. Within *C. neoformans*, two main lineages, which correspond to the current varieties *C. n.* var. *grubii* and *C. n.* var. *neoformans*, can be recognised by MultiLocus Sequence Typing (MLST) and Amplified Fragment Length Polymorphism (AFLP™) fingerprinting. In addition, diploid or aneuploid hybrids have been documented to have formed between these two varieties. Within *C. gattii* presently no infraspecific taxa are recognized. However, four distinct genotypic groups can be distinguished by MLST and AFLP. These lineages appear to be monophyletic, a finding that strongly calls question the current taxonomic handling of *C. gattii* into question.

Normally, *C. neoformans* and *C. gattii* reproduce asexually, but in some cases mating can occur. The existence of hybrids between *C. n.* var. *grubii* and *C. n.* var. *neoformans* demonstrates that mating occurs in nature. Recently, we have also documented a number of unique hybrids between *C. neoformans* and *C. gattii*. The recognition of the isolates involved as hybrids with this unexpected genetic background was supported by AFLP genotyping, sequence analysis of various genome domains after cloning of PCR amplicons, flow cytometry to assess the DNA content, fluorescence microscopy to investigate the number of nuclei, and analysis of the diversity in the InterGenic Spacer (IGS) by Luminex technology. All the data available suggested that these hybrids, which were isolated from Dutch patients, have originated from a mating between *C. gattii* AFLP genotype 4 and *C. n.* var. *neoformans* AFLP genotype 2. Interestingly, recently another hybrid type, a cross between *C. n.* var. *grubii* AFLP genotype 1 and *C. gattii* AFLP type 4, was obtained from Canada. The present data indicated that all these hybrids are diploid or aneuploid and may be unable to undergo a regular meiosis. If this



DNA microarray of Cryptococcus neoformans.

hypothesis is true, the species within the *Cr. neoformans* complex may be genetically separated by postzygotic reproduction barriers. Further research aims at assessing the biological species concept, in addition to the above-applied phylogenetic species concept, to determine the extent to which these concepts correspond with the biological realities seen within the complex.

A CBS-developed Agilent microarray, based on the genome of *C. neoformans* isolate JEC 21 (serotype D), has been used for comparative genome hybridisation experiments involving isolates belonging to all the genotypes known within the complex, as well as some of the known hybrids. The results confirm that *C. n.* var. *grubii* and *C. n.* var. *neoformans* are relatively closely related to each other, and are relatively distantly related to the four genotypes of *C. gattii*. Interestingly the hybridisation patterns of the AD hybrid differed widely from that of the BD hybrid, although in both cases only the serotype D genetic background was elucidated by the array. This may imply that considerable differences exist among the serotype D backgrounds found in the various hybrids, thus supporting the notion that the hybrids may be highly aneuploid.

Importantly, isolates of a genetic subgroup of *C. gattii* referred to as AFLP genotype 6 (= PCR-fingerprint group VGII) have been recognized as having caused a major cryptococcosis outbreak in Vancouver Island, Canada, which recently extended to the Canadian mainland, thus enlarging its area of distribution and gaining continental access. This outbreak mainly affects otherwise healthy people, but animals, including marine mammals can be affected. Ecological sampling has indicated that the same genotype occurs on many native tree species, which collectively may represent the main environmental reservoir involved in the outbreak. Recently, a Danish tourist who traveled to the part of Canada affected by the outbreak developed cryptococcal pneumonia. Detailed genotypic analysis demonstrated that the isolate obtained from this patient was identical to those from Vancouver Island. Hence, this investigation documented the first known tourist-mediated intercontinental transmission of this disease and pathogen.

To trace the origin of this ongoing outbreak in an area of northern temperate climate, we used comparative AFLP to search for novel, highly variable, molecular markers useful in development of an MLST scheme. Six DNA regions were selected to be sequenced for ca. 120 genotype AFLP 6 isolates, including many from the Vancouver outbreak. Preliminary data suggest that the Vancouver Island *C. gattii* outbreak may have been caused by extension of the South American *C. gattii* population and that recombination possibly played a role in the emergence of the hypervirulent subgenotype AFLP 6A implicated in the Vancouver Island outbreak. The mechanism that brought *C. gattii* northward is still unknown. However, it has been suggested that global warming may have influenced the distribution of this tropical yeast in Northern America.

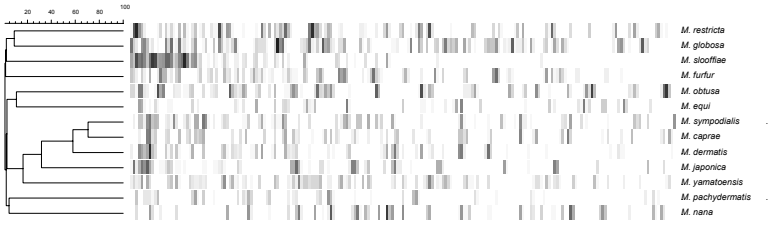
The pathogenicity of *C. gattii* genotype AFLP 6 was further studied using both the nematode worm *Caenorhabditis elegans* and a mouse model system (in collaboration with Dr. R. May, Birmingham, UK; I. Polacheck, Jerusalem, Israel). The absence of an adaptive immune system in *C. elegans* allows this model to be used to dissect out “basic” cryptococcal virulence factors. Interestingly, considerable differences were observed among isolates from the Vancouver Island outbreak that could not be distinguished otherwise. Ongoing research will aim to understand the basis of these differences. Various molecular and biochemical approaches will be used (in collaboration with U. Himmelreich, Cologne, Germany; G. Janbon, Paris, France, and F. Coenjaerts, University Medical Centre, Utrecht University).

A functional genomics analysis performed in collaboration with F. Coenjaerts concerned a serotype D strain of *C. n.* var. *neoformans* that had a mutant form of the Skn7 gene (listed by the *Saccharomyces* genome database as a nuclear response regulator and transcription factor required for optimal induction of heat-shock genes in response to oxidative stress). Preliminary data suggest that only few genes were upregulated after exposure of the mutant to oxidative stress. Further research will include a more detailed bioinformatics analysis, as well as additional genetics and microarray experiments.

Malassezia: *Malassezia* yeasts are associated with several dermatological disorders in both humans and animals. They are the causative agents of tinea (pityriasis) versicolor, and have also been suggested to trigger the immunological reactions involved in seborrheic dermatitis, atopic dermatitis, common dandruff and a number of other dermatological diseases. The precise role of the yeasts in all these conditions has not been fully elucidated. Until a few years ago, the genus *Malassezia* comprised only three species, but subsequently the number of species has risen to seven, and recently some more species have been found. In this research we aim to obtain a better understanding of the genus and its role as a pathogen.

The genetic diversity within the lipid-dependent species

AFLP tree of some new Malassezia *species.*

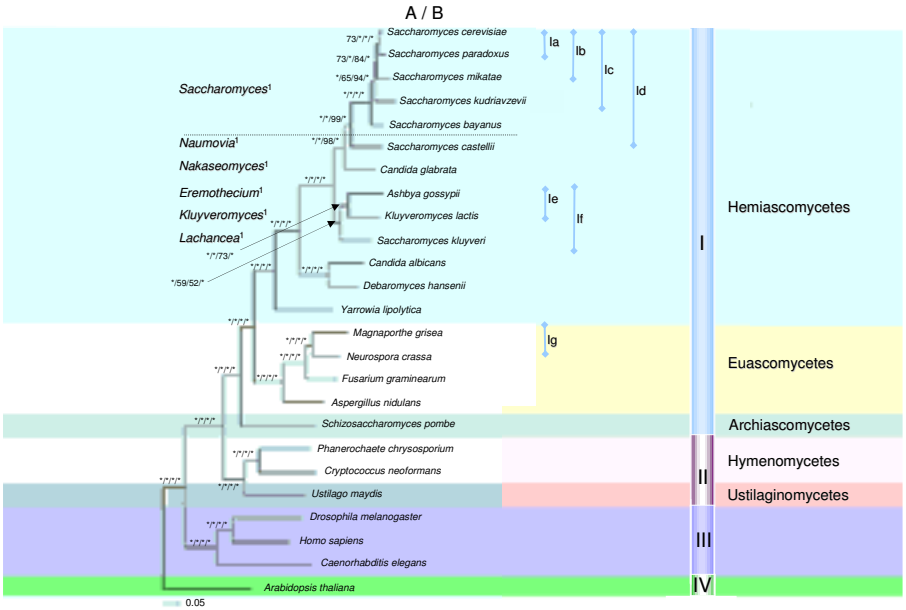


Malassezia furfur was investigated in collaboration with Roma Batra (Milwaukee, U.S.A.). In AFLP analysis, we found several subclusters within the species. Additional techniques have been used to analyse this complex further, e.g., pulsed field gel electrophoresis as well as sequencing of the LSU and ITS regions of the rDNA and part of the chitin synthase gene. As part of the results, one of the *M. furfur* subclusters was shown to have a mixture of markers suggestive of a hybrid origin, even though no sexual mechanisms are known so far for any species clustering within the *Malassezia* lineage.

In collaboration with Javier Cabañas (Barcelona, Spain) we analyzed lipid-dependent strains from different animal species and found three distinct clusters closely related but not identical to *Malassezia sympodialis*. Two of these clusters are presently in the process of being described as novel species. The third cluster represents *M. nana*, known from animals, but this species seems to be heterogeneous in its present circumscription and needs novel species definitions.

Other yeasts: Clinically important yeasts from neonates and HIV-infected persons were studied from Jakarta (Indonesia) in collaboration with Retno Wahyuningsih. Interestingly, the recently described species, *Candida nivariensis*, a close relative of *Candida glabrata*, was found. This is species has otherwise only been reported in connection with its original taxonomic description. Also *Candida ethanolytica* was identified from clinical sources, though it was not confirmed as an etiologic agent. This research was supported by a SPIN (KNAW) mobility grant.

A collection of *Trichosporon* isolates was identified using molecular tools in collaboration with Dr Saad J. Taj-Aldeen (Doha, Qatar). Most isolates could be readily linked to known species, but one isolate seems to represent a novel species from a clinical source.



Phylogenomic tree of fungi using full genome data.

Fungal Phylogenomics

Phylogenomics is the merger of three disciplines: genomics, the study of how genes are utilised to construct and maintain whole organisms; bioinformatics, the discipline handling complex biological information databases; and phylogeny, the study of how organisms have evolved. It is now possible to perform studies on fungal evolution using the data derived from whole-genome sequences of different species. Such large-scale projects demand extensive collaboration, and we have developed such collaborative studies with other research groups in the Netherlands such as those of B. Snel (Centre for Molecular and Biomolecular Informatics, University of Nijmegen), L. Stougie (CWI, Center of Mathematics and Informatics, Amsterdam) and C. Waalwijk and T. van der Lee (Plant Research International, Wageningen).

Phylogenetic studies are generally based on comparing DNA or protein sequences that, though found in a wide range of organisms, all arose from the same ancestral genes that occurred millions of years ago in a hypothetical common ancestor species. Such genes that occur among many organisms, but that all have a common ancestral root, are referred to as “orthologous” (= “directly related”). Orthologous groups of proteins (KOGs, or “euKaryotic clusters of Orthologous Groups of proteins”) from complete genomes of 19 different fungal species were analysed in order to resolve the phylogenetic relationships of these species. Phylogenomic analysis of unique fungal KOG’s resulted in well resolved and congruent phylogenetic trees. It appears that the *Basidiomycetes* occur as a sister group to the *Ascomycetes*. Within the latter, three lineages occurred, namely the basal *Archaeascomycetes* (represented by *Schizosaccharo-*

myces pombe), with the filamentous *Euascomycetes* and the yeast-like *Hemiascomycetes* arising as derived sister groups. The data also strongly suggest that the ancestor of these lineages was dimorphic, allowing maximal lifestyle flexibility for organisms coping with varying ecological conditions. In a separate study in which the individual KOG trees were compared using cluster analysis, distinct clusters of KOGs were found. When concatenated, most of them support the phylogenetic patterns described above. One, however, was found to yield a complete divergent tree topology. These results stimulated questions about how the fungi originated, deep in their evolutionary past.

A functional study of the septal pore caps in basidiomycetes

The septal pore cap (SPC) or parenthesome is a membranous structure associated with endoplasmic reticulum. It is situated on both sides of the dolipore septum and is restricted to certain groups of Basidiomycetes. Although this structure was already described in 1958 and well studied at an ultrastructural level, no functional studies have been done so far. Therefore, the composition and the precise function of the SPC at the dolipore is still unclear. The aim of this study was to isolate the SPC and to partly characterise the proteins present so that a start can be made in understanding the function of this part of the fungal cell. We used the plant pathogen *Rhizoctonia solani* as a model organism, because it has relatively well-studied, large SPCs.

Isolated septal pore cap of Rhizoctonia solani.

Laser microdissection with a P.A.L.M. microscope (P.A.L.M. Microlaser Technologies GmbH, Bernried, Germany) was used successfully to isolate the SPC-dolipore region. We could identify the septal regions using lectin-gold labelling of antibodies specifically targeting the septa; this analysis was done with a scanning electron microscope. In addition, we successfully enriched SPCs from *R. solani* cell fractions by isopycnic (= buoyant density or equilibrium) centrifugation. In electron microscopic studies, we observed that plug material at the orifice of the septal pore channel remained attached via filamentous material to the SPCs. This tight connection between SPCs and pore-occluding material implicates a key role of SPCs in the process of plugging septal pores in *Basidiomycetes*. Such plugging is often connected to maintaining hyphal integrity in situations where some cells are damaged or otherwise strongly stressed.

Protein electrophoresis showed that a 18 kDa glycoprotein (SPC18) was present in the SPC-enriched fraction. This protein was N-terminally sequenced and afterwards the complete gene sequence was obtained. No homologue could be identified using the available sequences in genome databases. Western blot analysis, however, suggests that the protein may be limited to the *R. solani* lineage. However, the SPC18 gene could be detected by PCR in a variety of other basidiomycetes. Attempts are ongoing to study the nucleotide diversity of the gene within the *Rhizoctonia* lineage and to compare it with standard D1/D2 variable region sequences of the 26S ribosomal DNA.

Polyclonal antibodies raised against the 18 kDa glycoprotein were labelled using the immunogold technique and then used to perform immunodetection studies. The labelled antibodies were found to be localized in the dolipore swelling as well at the SPC membrane and, to some extent, in the cytoplasm close to the SPCs.

The idea that SPCs play a role in plugging septal pores in *Basidiomycetes* made it interesting to study differential gene expression in cells exposed to a stress situation that would normally cause plugging of septal pores to occur. The mycelium was “stressed” in a blender and septal pores of the broken hyphae became plugged, as was easily seen using a fluorescence microscope. With the so-called ”suppression subtractive hybridisation” method for testing differential gene expression, we obtained sequences of genes, including those putatively involved in the plugging event, that were upregulated as a result of the short period of mechanical stress. Annotation of these sequences is in process and will lead to new insights in the mechanisms of the plugging process in *Basidiomycetes*.

The observed heterogeneity of SPC morphology in some of the major lineages of the *Basidiomycetes*, notable the *Hymenochaete* and *Cantha-rellus-Rhizoctonia* clades, was confirmed by analysing the SPC of *Rickenella* spp. and *Cantharellus* sp. It appeared that the observed heterogeneity in SPC morphology is characteristic of basal basidiomycete lineages, and extends only a short evolutionary distance into the derived lineages. This implies that genes involved in both types of SPC morphology may be present in these basal lineages, and that the basal organisms may thus manifest a genetic condition that existed in the ancestors of other *Basidiomycetes* prior to the occurrence of lineage sorting.

Applied and Industrial Mycology

Fungi play an important role in our daily life, both as agents of spoilage in food and feed and as agents of deterioration in building materials, artworks, museum objects, archives and a wide variety of other valuable items. Worldwide, the quantity of food products that is lost due to fungal spoilage is immense. This loss can be caused by post-harvest problems, in which fungi attack still-living fruits, vegetables and grains, but it can also arise in processed foods affected by spoilage fungi. The growth of fungi may result in off-flavours, discoloration and altered texture, all of which contribute to the conspicuous phenomenon of rot. What is not so readily visible but is much more alarming is the formation in some contaminated foods of fungal toxins (mycotoxins) or pathogenic or allergenic fungal spores. In addition to concerns about mycotoxins in foods, there is also currently increasing concern over the fungal growth within buildings. Although fungi are always present around us and cannot be eradicated totally, some aspects of their excessive presence in buildings can be linked to serious adverse health problems. Fundamental to investigating these applied research topics is to have a stable taxonomic classification, a reliable "who's who," of the organisms involved.

Aspergillus *conidiophore.*

Polyphasic taxonomy of the genera *Penicillium* and *Aspergillus*

The taxonomy of the mycotoxin-producing genera *Penicillium* and *Aspergillus* has been investigated for many decades and constitutes one of the central themes of the CBS, which has been doing taxonomic research on these genera since the 1940's. The taxonomic research of today is based on a strong interdisciplinary and integrated approach including study of morphology, biochemistry, physiology and molecular biology.

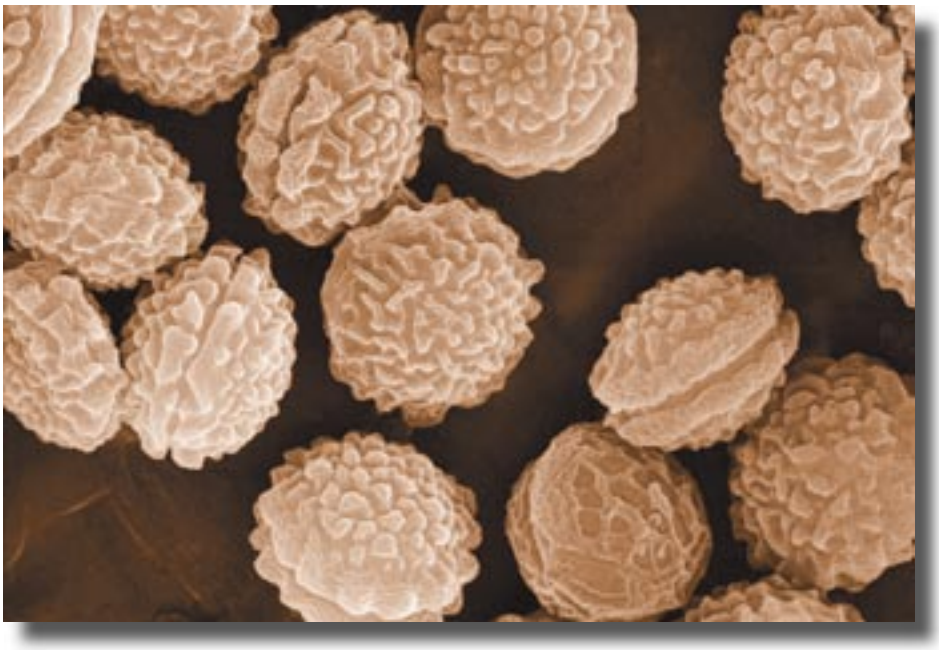
A major study comprising species in *Penicillium* subgenus *Penicillium* was completed. Many species belonging to this subgenus are very common, being associated with foods stored by humans and animals, and also growing on animal dung, building materials, and numerous other types of natural and human-made materials. Since many of these materials are found in indoor environments, these *Penicillium* species provide a major component of the indoor air spora. The taxonomy of this group was long recognized as being especially difficult, but the advent of molecular techniques and refined study of metabolite profiles allowed a stable taxonomy of these species to be proposed. It was based on a polyphasic study of a large number of isolates, and ultimately accepted 58 species. Four new species, *P. cavernicola, P. freii, P. marinum* and *P. thymicola*, were described, and two new taxonomic combinations were made yielding the names *P. melanoconidium* and *P. neoechinulatum.* The species were ordered in natural sections and series. At the level of series, the groups recognised were not just phylogenetically but also ecologically consistent.

Descriptions and colour illustrations of the colonies and micromorphology of the 58 accepted species were included in the *Studies in Mycology* volume in which this work was published. Keys to the taxa were provided. For the additional help of those doing identifications in this group, a detailed electronic database including partial β-tubulin sequences reference was set up at http: //www.cbs.knaw.nl/penicillium.htm.

In *Aspergillus*, several taxonomic sections of the genus were studied, including section *Circumdati, Nigri, Flavi* and *Fumigati*. These sections were selected for investigation because they include taxa playing a significant role as food contaminants, mycotoxin producers, and opportunistic human and animal pathogens. Polyphasic analysis revealed a clear-cut delimitation of species, including several new taxa. In *Aspergillus* section *Fumigati*, the taxonomic analysis isolates identified as the commonly occurring compost inhabitant and opportunistic pathogen *A. fumigatus* yielded three undescribed taxa highly morphologically similar to *A. fumigatus*, which were described as *A. lentulus, A. fumigatiaffinis* and *A. novofumigatus*. The steadily increasing scientific and medical interest in members of section *Fumigati*, evidenced, for example, by the production of a complete genome sequence for *A. fumigatus*, demonstrates the need for a stable taxonomy defining the distinct biological groups within this evolutionary lineage.

Food mycology

Currently, there is a strong demand for fresh food products and a preference that these products contain no added preservative substances. This quest for healthy foods paradoxically has tended to stimulate a novel upsurge in fungal spoilage incidents. In general, several strategies for food preparation can make it difficult for fungi to cause contamination. Long-standing techniques include controlling water activity through drying or by adding salt or sugar, using pasteurization, canning or other types of heat treatment, and using storage conditions unfavourable to fungi, such as low temperatures or acidic materials such as vinegar. Some fungi, however, are resistant to these traditional preservation methods. To fight these specialised food spoilage fungi, food preservatives are added, but even with these materials, resistant fungi may occur. In connection with these problems, research has been initiated at CBS to evaluate possible new food preservatives and to determine their influence on fungal cells.



Ascopores of undescribed Neosartorya *species.*

Heat-resistant ascospores

Research on heat-resistant ascospores is one of the lines of research at Applied and Industrial Mycology. At CBS, the fungus *Talaromyces macrosporus* is used as a model system to study the biology of heat resistant ascospores in detail. These spores allow the fungus to survive heat treatment of foods; indeed, they often remain dormant until high temperatures stimulate them to germinate. Various molecular and microscopic tools are used to unravel the mechanisms that cause the extraordinary stress resistance of the ascospores. This has resulted in a number of papers on this subject, and the research will be continued in the near future. Current projects include collaborations with Molecular Microbiology at the University of Utrecht (Drs Han Wösten and Luis Lugones) and with Drs. Golovina and Hoekstra at Plant Physiology of the Unversity of Wageningen. At present, research on the physical properties of the cytoplasm of *Talaromyces* ascospores is being conducted by means of electron spin-resonance studies. It has become clear that ascospores of *T. macrosporus* have a very high viscosity, much higher than that observed in airborne conidia of *Penicillium* species. During ascospore germination, sudden changes in viscosity occur; in conidia, by contrast, a more gradual change occurs. Conidia and ascospores clearly differ markedly in cytoplasmic structure and germination properties.

Further research has dealt with the mechanisms of ascospore dormancy as well as those regulating heat activation. In collaboration with the University

Penicillium *contamination of cheese*.

of Utrecht, we have identified a highly distinctive protein that is abundantly present inside ascospore cell walls; further research is being directed at elucidating its function.

Inititiation of an STW-funded project for CBS

An STW-funded project entitled "Natamycin as a perturbator of the fungal membrane" was awarded in May 2004. The project is being led by Jan Dijksterhuis and includes the cooperation of Drs Eef Jan Breukink and Ben de Kruijff from the Institute for Biomembranes of the University of Utrecht. In February 2005, Richard van Leeuwen started his Ph.D research on the mechanism of action of the compound natamycin on the fungal membrane.

The project is a direct result of previous work of Jos Houbraken, Ellen Hoekstra and Rob Samson on the effect of natamycin on different species of fungi including *Penicillium discolor*, which is an important spoilage organism of cheese surfaces.

To prevent the growth of *P. discolor* and some other fungi, natamycin is regularly used in coatings for cheeses and sausages. DSM, the main industrial producer of natamycin in the world, is an important collaborator in the project. Various stakeholders, including DSM, felt that it was necessary to learn more about the mode of action of natamycin, a matter about which little is known. The compound is known to bind to ergosterol, a major sterol component of the fungal membrane, but it may differ in mode of action from related polyene compounds that also bind to ergosterol. Nystatin, for example, binds to ergosterol to form pores that cause leakage of cell constituents, resulting in cell death. Ergosterol and related sterols were long thought to be associated with an increase of the fluidity of the membrane during low temperatures or periods of salt stress, but novel insights hint that these compounds may have additional important functions, for example in the organising the plasma membrane and in the trafficking of membrane vesicles into the cells.

Germinating conidia of Penicillium discolor.

The project also focuses on the influence of polyene antibiotics on spore germination, Spores play an important role in the airborne distribution of the fungi involved in cheese surface spoilage.

CBS plays an important role in a large Senter-project

An important Senter project was awarded to a consortium of Dutch partners including PPO (Applied Plant Research) and PRI (Plant Research International) as well as industrial partners DSM, Holland Fyto and Innoventis. CBS plays an important role in the fungal aspects of this project. The project grew out of pilot projects done in 2003–2004 with DSM and PRI. The research is on the plant pathogenic fungus *Fusarium oxysporum*, which can infect and destroy tulip bulbs at different stages of processing. The fungus enters the bulbs via small wounds caused by handling

Fungal infections cause massive losses of flowerbulbs.

or via openings naturally created by the emerging roots during bulb development. The losses caused by this fungus can be enormous and only very strong chemical compounds are able to counteract the infection. This project arose from the desire to develop a more environmentally friendly mode of disease control. Fundamental knowledge about the growth of the fungus, both under optimal conditions and in the presence of various inhibitory compounds and compound mixtures will be acquired. It is hoped that the results can quickly be translated into amenable disease control techniques.

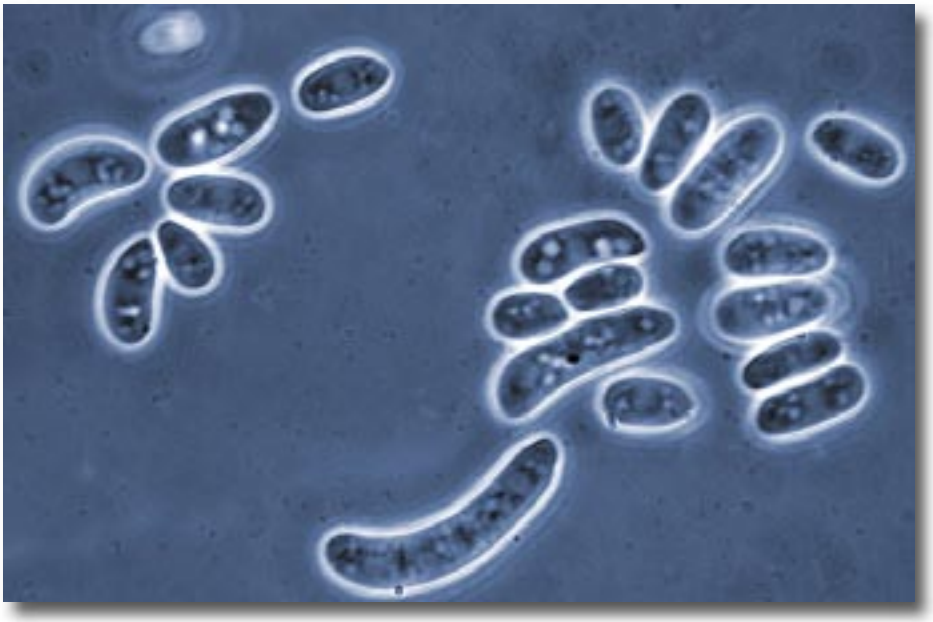
Fungi in indoor environments

In investigations of fungi in indoor situations, the principal objectives of the investigators are to detect sources of excess moisture stimulating fungal growth, to measure the fungal load present indoors, and to quantify the exposure of building occupants to fungi. Many fungi proliferating indoors have potentially toxic, irritating, or allergenic metabolites and cell wall components. Contrary to what occurs in other areas of mycological research, in this area methodologies and interpretations may vary considerably in different countries and even among different laboratories. This variation often makes direct comparison of results impossible. From the medical as well as the building construction standpoint, the need to establish international standards for sampling protocols and results interpretation is strongly felt.

The purpose of the international workshop organised by Rob Samson, Olaf Adan (TNO, Delft) and Nicole Nolard (Brussels, Belgium) and held on March 15–17, 2005, was to bring together experts in mycology, respiratory health, building material science and building construction to discuss the state of the art related to this topic. Thirty-six participants from Belgium, Denmark, Canada, Finland, France, the Netherlands, U.K. and the U.S.A. attended. Besides various microbiological aspects of fungal growth and health implications, emphasis was given to the factors favouring growth of fungi in domestic environments. Opinions were presented about how eco-friendly trends such as increased energy conservation, leading to decreased indoor air exchange and thus an indoor humidity buildup, could be reconciled with the prevention of microbial proliferation on building materials. Presentations, recorded discussions and recommendations from this workshop in the process of publication. Recommendations made by the international platform will also appear at www.indoormold.org.

Aspergillus versicolor, *a common indoor contaminant*.

CBS researchers are continuing to develop a proficiency testing program designed to ensure good quality identifications in laboratories offering mycological expertise in relation to fungal problems in buildings. This project is a collaboration with German research groups in Stuttgart and Lübeck. Guidelines for detecting, identifying and handling indoor fungi have been published in cooperation with the Landesgesundheitsamt Baden-Württemberg (State Health Office of Baden-Württemberg) in Germany.



Fusarium oxysporum *f.s.* tulipae.

Programmes, Themes and Projects

(for detailed descriptions of the programmes, themes and projects, consult our website)

1. Yeast and Basidiomycete Research (T. Boekhout)

Theme: Evolutionary genomics of fungi

Project YBRP 1.01.01: Comparative Fungal genomics
2003–2007: **T. Boekhout**, **E. Kuramae (postdoc),** B. Snel (Bio-informatics, Utrecht University, The Netherlands), L. Stougie, P. Vitanyi & R. Cilibrasi (CWI, University of Amsterdam, The Netherlands), T. van der Lee, C. Waalwijk, G. kema, R. van der Ham, J. Leunissen, (PRI, Wageningen University, Wageningen, The Netherlands), A. van der Burgt (aio PRI), Arnold Kuzniar (aio Wageningen University), M. Weiss, University Tübingen, Germany).
Funding: KNAW Renewal fund, Genomics fund.

Project YBRP 1.01.02: Functional diversity of human pathogenic yeasts (including evolution, virulence and pathogenesis)
2003–2007: **T. Boekhout, E. Kuramae (postdoc), V. Robert, B. Theelen, F. Hagen**, **M. Bovers (Ph. D. student),** I. Hoepelman, F. Coenjaerts (Department of Internal Medicine and Infectious Diseases, UMC, Utrecht University, The Netherlands), R. May (University of Birmingham, UK), H. Hoogveld (NIOO-KNAW, Nieuwersluis, The Netherlands), E.J. Kuijper Department of Medical Microbiology, (LUMC, University of Leiden, The Netherlands), L. Spanjaard, (Department of Medical Microbiology, AMC, University of Amsterdam, The Netherlands), F. Hochstenbach (Department of Biochemistry, AMC, University of Amsterdam, The Netherlands), M. Lazera (Fundaçao Oswaldo Cruz, Brazil), C. Paula (University of São Paulo, Brazil), I. Polacheck (Hadassah Medical Centre, Israel), J. Heitman (Duke University, USA), W. Meyer (University of Sydney, Australia), U. Himmelreich (MPI, Cologne, Germany), G. Janbon (Institut Pasteur, Paris, France), S. Oliver (University of Manchester, UK), J. Fell & M. Diaz, (RSMAS, University of Miami, USA), R. Wahyuningsih (Schools of Medicine, Universitas Indonesia and Universitas Kristen Indonesia, Jakarta, Indonesia), A. Botha (University of Stellenbosch, S. Africa), F.J. Cabañes, Autonomous University of Barcelona, Spain), T. Dawson (Procter & Gamble, USA), R. Batra (Milwauki, USA), E. Guého (Mauves sur Huisnes, France), L. Ball (LUMC, University Leiden, The Netherlands), H. Korporaal (Leids Cytologisch en Pathologisch Laboratorium, Leiden, The Netherlands), S. Taj-Aldeen (Hamad Medical Corporation, Doha, Qatar), I. Okoli (Awka, Nigeria).
Funding: Odo van Vloten, KNAW Renewal Fund, KNAW Indonesia-Netherlands SPIN mobility grant.

Project YBRP 1.01.03: Biodiversity of yeasts and selected basidiomycetes

Project YBRP 1.01.03.01: 'The yeasts, a taxonomic study 5th edition'
2005–2007: **T. Boekhout, V. Robert,** J.W. Fell, G. Scorzetti (RSMAS, Miami, USA), C.P. Kurtzman (USDA-NCAUR, Peoria, USA), T. Nakase (NITE, Tokyo, Japan), M. Hamamoto (Meiji University, Higashimita, Japan), A. Fonseca, J.P. Sampaio (New University of Lisbon, Caparica, Portugal), R.J. Bandoni (Vancouver, Canada), F.J. Bai (Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, The Chinese Academy of Sciences, Beijing, China).

Project YBRP 1.01.03.02: 'Studies on the microbiological and biochemical properties of *masau* (*Ziziphus mauritiana*) fruits fermentation and prospects for the development of starter cultures to produce *masau* wine and/or beverage'
2004-2008: **T. Boekhout**, L. Nyanga (Ph.D student Wageningen University), R. Nout, M. Zwietering (Laboratory of Food Microbiology, Wageningen University, The Netherlands).
Funding: McGillavry fund, International Foundation of Science.

Project YBRP 1.01.03.03: The septal pore complex (SPC) in Basidiomycetes (*Rhizoctonia solani*)
2003–2007: **T. Boekhout**, **E.E. Kuramae, K.G.A. van Driel** (Ph.D. student), W.H. Müller & A. Verkleij (Department of Molecular Cell Biology, Utrecht University, Utrecht, The Netherlands), H. Wösten & A.F. van Peer (Department of Molecular Microbiology, Utrecht University, The Netherlands), A. Nakatani (PhD student, UNESP, São Paulo, Brazil), D. Rosa (M.Sc student, USP, Piracicaba-SP, Brazil).
Funding: Odo van Vloten, Utrecht University

Project YBRP 1.01.03.04: The *Rhizoctonia solani* Tree of Life.
2005–2006: **T. Boekhout**, **E.E. Kuramae, J.A. Stalpers, K.G.A. van Driel (Ph.D. student),** A. Nakatani (PhD student, UNESP, São Paulo, Brazil), N. de Souza (UNESP, Botocatu, Brazil),
Funding: CNPq (Brazil).

Project YBRP 1.01.04.05: Fungal biodiversity in regenerating tropical lowland forests, Colombia.
2002–2006: **T. Boekhout**, **R. Summerbell, C. Lopez-Q. (Ph.D student)** (CBS / Universidad de Antioquia, Medellin, Colombia), A. M. Cleef, J. Duivenvoorden & J. Sevink (University of Amsterdam, The Netherlands), A.E. Franco Molano & A. Vasco-P. (University of Antioquia, Colombia), J. Frisvad (Technical University, Denmark).
Funding: NWO-Wotro.

2. Applied and Industrial Mycology (R.A. Samson)

Theme 1: Biodiversity and ecology of food and airborne fungi

Theme 2: Biology of food spoilage fungi

Project IFA 2.01.01: Biodiversity of *Penicillium*, *Aspergillus* and related genera
2003–2010: **R. Samson**, **E. Hoekstra**, **J. Houbraken**, J.C. Frisvad (Lyngby, Denmark), K.A. Seifert (Ottawa, Canada).

Project IFA 2.01.02: Biodiversity and strain selection of fungi in indoor environments for quality management
2003–2007: **R.A. Samson, E.S. Hoekstra,** T. Gabrio, (Landes Gesundheitsamt Baden-Wurttemberg, Stuttgart, Germany), K. Senkpiel, R. Keller (Medizinischer Universität zu Lübeck, Germany).

Project IFA 2.01.03: Taxonomy and phylogeny of food borne Zygomycetes
2003–2007: **R. Samson, J. Dijksterhuis, A. Kuijpers, J. Houbraken**, J. Jenneson, J. Schnurer (University of Agriculture, Uppsala, Sweden).

Project IFA 2.02.04: Image analysis macroconidia of *Fusarium culmorum*
2002–2005: **J. Dijksterhuis**, G. Chitarra, T. Abee, F. Rombouts (Food Microbiology, University of Wageningen, The Netherlands).

Project IFA 2.02.05: Volatile compounds as germination regulators in *Penicillium paneum*
2003–2005: **J. Dijksterhuis**, G. Chitarra, T. Abee, F. Rombouts (Food Microbiology, University of Wageningen, The Netherlands).

Project IFA 2.02.06: Germination of heat resistant ascospores of *Talaromyces macrosporus*
2003–2006: **J. Dijksterhuis**, F. Hoekstra, E. Golovina, J. Nijsse (Plant Physiology, University of Wageningen, The Netherlands)**.**

Project IFA 2.02.07: The cell wall of ascospores of *Talaromyces macrosporus* before and after heat activation
2003–2006: **J. Dijksterhuis, M. Hanssen, T.T. Wyatt,** L. Lugones, H Wösten (Molecular Microbiology, University of Utrecht, The Netherlands), J.H. Sietsma (Molecular Biology of Plants, University of Groningen, The Netherlands).

Project IFA 2.02.08: Role of natamycin as a membrane pertubator in fungal conidia and hyphae
2005–2008: **J. Dijksterhuis, R.A. Samson,** E.J. Breukink, B. de Kruijf (Biomembranes, University of Utrecht, The Netherlands).

Project IFA 2.02.09: Sustainable control of fungal diseases of flower bulbs.
2005–2008: **J. Dijksterhuis, J. Houbraken, T. van Doorn,** W. van der Krieken (PRI, Wageningen), A. van der Lans, M. de Boer (PPO, Lisse), J. Stark, F. van Rijn (DSM, Delft), H. de Vries (Innoventis, Breezand), G. Top (Profyto, Emmeloord).

3. Evolutionary Phytopathology (P.W. Crous)

Theme 1: Evolutionary patterns and host adaptation

Theme 2: Mating strategies and speciation

Project EPP 3.01.01: Hybridisation in *Phytophthora*
2001–2005: **A.W.A.M. de Cock**, W.A. Man in 't Veld (Plant Protection Service, Wageningen), C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada).

Project EPP 3.01.02: Genetics of host specificity and speciation within *Cercospora*, with specific reference to *C. beticola*
2003–2006: **P.W. Crous, E.C.A. Abeln, M. Groenewald** (Ph.D. student), P. de Witt (Phytopathology, University of Wageningen, The Netherlands), H. Sneider (IRS, The Netherlands).

Project EPP 3.01.03: Genetic diversity of *Mycosphaerella* species associated with Sigatoka disease on bananas
2003–2007: **E.C.A. Abeln, P.W. Crous, M. Arzanlou** (Ph.D. student)**,** G. Kema (Plant Research International, The Netherlands), J. Carlier (CIRAD, France).

Project EPP 3.02.04: *Mycosphaerella* spp. occurring on *Eucalyptus*
2003–2006: **P.W. Crous, J.Z. Groenewald**, G. Hunter (Ph.D. student), M.J. Wingfield, B.D. Wingfield, T. Coutinho (University of Pretoria, South Africa).
Funding: NRF, TPCP, University of Pretoria, South Africa.

Project EPP 3.02.05: Speciation in *Cercospora*
2003–2007: **P.W. Crous**, **J.Z. Groenewald,** U. Braun (Martin-Luther University, Germany), H-D. Shin (Korea University, Seoul).
Funding: Volkswagenstiftung.

Project EPP 3.02.06: Circumscription and detection of the Cylindrocarpon black foot rot complex of grapevines
2002–2005: **P.W. Crous, H.-J. Schroers,** F. Halleen (Ph.D. student), P.H. Fourie (University of Stellenbosch, South Africa).
Funding: Winetech, ARC-Nietvoorbij, South Africa.

Project EPP 3.02.07: Developing microsatellite markers for *Cylindrocladium*
2003–2006: **P.W. Crous,** B. Buthelezi (M.Sc. student), L. Wright (Ph.D. student), M.J. Wingfield, B.D. Wingfield, T. Coutinho (University of Pretoria, South Africa).
Funding: TPCP, NRF, South Africa.

Project EPP 3.02.08: Colletotrichum anthracnose of *Proteaceae*
2002–2004: **P.W. Crous, J.Z. Groenewald,** K. Lubbe (M.Sc. student), S. Denman (Forestry Commission, UK), S. Lamprechts (University of Stellenbosch, South Africa), P. Cannon (CABI, UK).
Funding: SAPPEX, ARC-Elsenburg, South Africa.

Project EPP 3.02.09: Circumscription and detection of *Phaeoacremonium* and *Phaeomoniella* in grapevines
2003–2006: **P.W. Crous, L. Mostert** (Ph.D. student), **E.C.A. Abeln, W. Gams, R.A. Summerbell & J.Z. Groenewald**.
Funding: Odo van Vloten.

Project EPP 3.02.10: Circumscription, detection and infection strategies of *Botryosphaeria* spp. in grapevines
2003–2006: **P.W. Crous, J.Z. Groenewald,** J. van Niekerk (M.Sc. student), P.H. Fourie (University of

Stellenbosch, South Africa), F. Halleen (ARC-Nietvoorbij, South Africa).
Funding: Winetech, NRF, South Africa.

Project EPP 3.02.11: Phylogeny and population genetics of *Alternaria* and related *Pleosporales*
2002–2006: **G.S. de Hoog**, **P.W. Crous,** B.M. Pryor (Tucson, USA), T.L. Peever (University of Washington State, USA), E.G. Simmons (Amherst, USA), B. Anderson (Lyngby Technical University, Denmark).

Project EPP 3.02.12: Phylogeny in the genus *Pythium* and development of a molecular identification and detection system
1996–2006: **A.W.A.M. de Cock**, C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada).

Project EPP 3.02.13: Species delimitation in *Pythium*
1996–2006: **A.W.A.M. de Cock**, G.R. Klassen, J.E.J.Bedard, A.M. Schurko (University of Manitoba, Winnipeg, Canada).

Project EPP 3.02.14: Phylogeny in the genus *Phytophthora* and development of a molecular identification and detection system
2001–2007: **A.W.A.M. de Cock**, C.A. Lévesque (Agriculture and Agri-Food, Ottawa, Canada), R.C. Hamelin, G.Bilodeau (Natural Resources Canada, Canadian Forest Service, Québec, Canada).

Project EPP 3.02.15: Species delimitation in *Phytophthora*
2001–2007: **A.W.A.M. de Cock**, G.R. Klassen, J.E.J. Bedard, A.M. Schurko (University of Manitoba, Winnipeg, Canada), Man in 't Veld, W.A. (Plant Protection Service, Wageningen, The Netherlands).

Project EPP 3.02.16: Taxonomy, phylogeny and biology of *Cladosporium*
2003–2007: **P.W. Crous, H.J. Schroers, G.S. de Hoog, R.A. Samson,** P. Zalar (PhD student), U. Braun (Martin-Luther University, Germany), J. David (CABI, UK), F. Dugan (Washington State University, USA).

Project EPP 3.02.17: Phytopathogenic *Phoma* complexes
2002–2005: **P.W. Crous, M. Aveskamp** (Ph.D. student), **G. Verkley**, **R.A. Summerbell, J.Z. Groenewald, J. de Gruyter** (Ph.D. student; PPS, Wageningen, The Netherlands), S.T. Koike, K. Subbarao (University of California, USA), T. O'Neill (ADAS, UK).

Project EPP 3.02.18: Taxonomy and phylogeny of *Septoria*
2000–2006: **G. Verkley, M. Starink-Willemse, A. van Iperen**, S. Vanev (Botanical Institute, Sofia).

Project EPP 3.02.19: Novel and putative ascomycetous plant endophytes
2003–2006: **G. Verkley, I. van Kempen, A. Aptroot, R.C. Summerbell**, J.D. Zijlstra, F. Berendse (University of Wageningen, The Netherlands).

Project EPP 3.02.20: Worldwide biodiversity of the genus *Mycosphaerella*
1998–2005: **A. Aptroot, P.W. Crous**, J. David (CABI, UK).

4. Origins of pathogenicity in clinical fungi (G.S. de Hoog)

Theme 1: Evolution and host adaptation of black yeasts and allied fungi

Theme 2: Extremophilic fungi

Project OPC 4.01.01: Natural life cycle and selective sweeps of *Exophiala dermatitidis*
2002–2006: **G.S. de Hoog, A.H.G. Gerrits van den Ende, M. Sudhadham** (Ph.D. student), T. Matos (Fac. Medicine, Slovenia), S. Sivichai (Chulalongkorn Univ., Thailand), G. Haase (RWTH-Aachen, Germany), G. Dorrestein (University of Utrecht, The Netherlands), A. van Belkum (Bacteriology, Erasmus Univ., Rotterdam), S.B.J. Menken (IBED, Amsterdam, The Netherlands), Y. Gräser (Charité, Berlin, Germany), A. Mayr (Hautklinik, Univ. Innsbruck, Austria), D. Jonkers (Alterra, Wageningen, The Netherlands).
Funding: WOTRO.

Project OPC 4.01.02: Taxonomy and antimycotic susceptibility of herpotrichiellaceous black yeasts
2002–2008: **G.S. de Hoog**, R. Vitale (Radboud, Nijmegen), G. Haase (RWTH-Aachen, Germany), V. Vicente (Microbiol., Univ. of Curitiba, Brazil), R. Caligiorne (Musée d'Histoire Naturelle, Paris, France), S. Kantarcioglu (Fac. Medicine, Istanbul, Turkey), F. Zeppenfeldt, Univ. Nac. Experim., Coro, Venezuela).

Project OPC 4.01.03: Black oligotrophs in indoor water systems and their impact on human health
2002–2006: **G.S. de Hoog, A.H.G. Gerrits van den Ende, J. Dijksterhuis, R.A. Samson, J. Harrak** (PhD student), S.B.J. Menken (IBED, University of Amsterdam, The Netherlands), A. van Belkum (Bacteriology, Erasmus University, Rotterdam, The Netherlands), E. Göttlich (LUFA Augustenberg, Karlsruhe, Germany), A. Velegraki (Fac. Medicine, Athens, Greece), N. Hageskal (Vet. Faculty, Oslo, Norway).
Funding: CBS/IBED.

Project OPC 4.01.04: Development of environmental test systems using fungal indicators
2002–2004: **G.S. de Hoog, W. Becker,** K. Verstraten (IBED, University of Amsterdam, The Netherlands), N.A. Yurlova (St. Petersburg, Russia), K. Groenestein (University of Wageningen, The Netherlands), J. Rainer (Botanik, Univ. Innsbruck, Austria), H. Nelis (Gent, Belgium), M. Richardson (Helsinki, Finland).
Funding: EU.

Project OPC 4.01.05: Infection and resistance of therapy-refractory emerging fungal opportunists in humans
2002–2006: **G.S. de Hoog**, M. Sabelis (IBED, University of Amsterdam, The Netherlands), S.B.J. Menken (IBED, University of Amsterdam, The Netherlands), J. Rainer (Inst. Botanik, Univ. Innsbruck, Austria), Y. Gräser (Charité, Berlin, Germany), J.-P. Bouchara (Clin. Dermatol., Angers, France), and

a 25-lab network composing an ECMM working group.
Partial funding: ECMM.

Project OPC 4.01.06: Agents of human mycetoma
2002–2004: **G.S. de Hoog, K.F. Luijsterburg,** A.O.A. Ahmed (Mycetoma Res. Center, Khartoum, Sudan), A. van Belkum (Bacteriology, Erasmus University, Rotterdam, The Netherlands).
Partial Funding: ISHAM Working Group Mycetoma.

Project OPC 4.01.07: Atlas of Clinical Fungi
2002–2008: **G.S. de Hoog**, **K.F. Luijsterburg,** J. Guarro, J. Gené, M.J. Figueras (University Rovira i Virgili, Reus, Spain), J. Albert (Inst. Informatik, Würzburg, Germany), D. Harmsen (Med. Mikrobiol. University of Münster, Germany).

Project OPC 4.01.08: Phylogenetic position and taxonomy of *Ochroconis* and *Scolecobasidium*
2002–2004: **G.S. de Hoog**, R. Horré (Bundesanst. Arzneimittel, Bonn, Germany), H.-J. Choi (Mikrobiol. Univ. Bonn, Germany).

Project OPC 4.01.09: Evolution of virulence in black yeasts
2004–2007: **G.S. de Hoog,** A. Ram (University of Leiden, The Netherlands), S.B.J. Menken (IBED, University of Amsterdam, The Netherlands), Jingsi Zeng, Shuwen Deng, Paride Abliz, Dongming Li, Ruoyu Li (Beijng / Xinjiang, P.R. China),
Funding: KNAW and Chinese Academy.

Project OPC 4.01.10: Evolution of extremotolerant black yeasts.
2002–2004: **G.S. de Hoog, A.H.G. Gerrits van den Ende, T. Ruibal,** N.A. Yurlova (St. Petersburg, Russia), N. Gunde-Cimerman (Ljublana, Slovenia), M. Grube (Graz, Austria), L. Selbmann (Viterbo, Italy), F. Lutzoni (Duke, U.S.A.)
Partial funding: AFTOL U.S.A.

Project OPC 4.01.11: Evolution of halophily in dothideaceous black yeasts
2002–2004: **G.S. de Hoog**, **A.H.G. Gerrits van den Ende, P. Zalar** (Ph.D. student; Inst. Biochemistry, Ljubljana, Slovenia).

Project OPC 4.01.13: Assimilation of toxic degradation products from lignin and oils by black yeast-like fungi
2003–2006: **G.S. de Hoog, F. Prenafeta, R.C. Summerbell, J. Dijksterhuis, M. Sudhadham, J. Harrak** (PhD student), K. Verstraten, K. Nierop (IBED, University of Amsterdam, The Netherlands), U. Hölker (Inst. Botanik Uni-Bonn, Germany), G. Haase (RWTH-Aachen, Germany), P. Letitre (MEP-TNO, Apeldoorn, The Netherlands), C. van den Hondel (CIVO-TNO, Zeist, The Netherlands).

Project OPC 4.01.14: Fungal-bacterial interactions in soil
2002–2006: **R. Summerbell**, **F. Prenafeta, T. Boekhout**, **A. van Iperen,** W. de Boer, G. Kowalchuk (NIOO-KNAW, The Netherlands).
Funding: KNAW Vernieuwingsfonds.

Project OPC 4.02.17: *Geotrichum*, a fungal dinosaur
2002–2004: **G.S. de Hoog, M.Th. Smith, A.W.A.M. de Cock,** C.P. Kurtzman (USDA, Peoria, USA), K. Ueda-Nishimura (Inst. Fermentation, Osaka, Japan).

Project OPC 4.02.18: The biodiversity of para-Hypocrealean fungi in human and animal disease
2002–2004: **R.C. Summerbell, H.-J. Schroers, M. Starink-Willemse, P.W. Crous**, **W. Gams**, **G.S. de Hoog**, **L. Mostert** (Ph.D. student), L. Sigler (University of Alberta, Canada), A.A. Padhye, M. Brandt (CDC, USA), S. Moser (Univ. Alabama), P. Kammeyer (Loyola Univ. Med Ctr, Maywood IL, USA), D. Sutton, M.G. Rinaldi (Fungus Testing Center, San Antonio, USA), W. Merz (Johns Hopkins, Baltimore USA), M. Hayden (Rush Med. Ctr., Chicago, USA), A. Goldschmit-Reuveni, G. Rahav (Tel Hashomer Hospital, Tel-Aviv, Israel), S. Krajden (St. Joseph's Hospital, Toronto, Canada).

Project OPC 4.02.19: Sequencing black yeast floras from human patients
2004–2006: **G.S. de Hoog, Jingsi Zeng,** D.A. Sutton (Fungus Testing Lab, San Antonio, U.S.A.)
Funding: Pfizer U.S.A.

5. Collection, Preservation and Digitalisation (J. Stalpers)

Theme 1: Preservation research

Theme 2: Sequencing and characterisation of type strains

Theme 3: Online access to nomenclatural and taxonomic databases

Project CPD 6.01.01: Optimalisation of freeze-drying protocols
2003–2005: **C.S. Tan**, C. van Ingen (RIVM, Utrecht, The Netherlands), C. van den Berg, R. Hoekstra (University of Wageningen, The Netherlands).

Project CPD 6.01.02: Freezing of recalcitrant organisms
2003–2004: **C.S. Tan, IJ. Vlug**.

Project CPD 6.02.01: Sequencing and characterisation of ex-type strains
2003–2010: **J.A. Stalpers, C.S. Tan**, **G. Verkley, G.S. de Hoog, R.A. Samson, W. Haisma, IJ. Vlug, A. Kuijpers, P.W. Crous, R.S. Summerbell, T. Boekhout.**
Funding: EU-EBRCN project.

Project CPD 6.03.01: Digitalisation and accessibility of nomenclatural and taxonomical data
2003–2010: **V. Robert, J.A. Stalpers, G. Stegehuis,** P. Romano (ABC, Italy).

Project CPD 6.03.02: Index of Fungi
2003–2006: **J.A. Stalpers, G. Stegehuis**, P. Kirk (CABI Bioscience, UK), J. Adams (Landcare, New Zealand).

Project CPD 6.03.03: Input of CBS data in CBS database
The databases will be linked with other, non-CBS databases, as PubMed and GenBank
2003–2005: **J.A. Stalpers, G. Stegehuis, V. Robert, M. Vermaas,** P. Romano (ABC, Italy).

Project CPD 6.03.04: CBS publications on the Web
2003–2005: **J.A. Stalpers, G. Stegehuis, D. Stalpers**.

Project CPD 6.03.05: European Biological Research Centres Network (EBRCN)
2001–2004: **J.A. Stalpers, G. Stegehuis**, D. Smith (CABI, UK), E. Stackebrand (DSMZ, Germany), C. Bizet (Institut Pasteur, France), P. Romano (ABC, Italy), D. Janssens (LMG, Belgium).
Funding: EU.

Project CPD 6.03.06: MOSAICS, Implementation of Access and Benefit Sharing related to Microbiological Resources
2004–2005**. J.A. Stalpers**, **G. Verkley,** D. Smith (CABI, UK) E. Stackebrand (DSMZ, Germany), C. Bizet (Institut Pasteur, France), B. Parodi (ABC, Italy), Ph. Desmeth (BCCM, Belgium).
Funding: EU

Project CPD 6.03.07: Eurocat, Species 2000 production c.q. improvement of the Catalogue of Life
2003–2006. **J.A. Stalpers, G. Stegehuis**, major European taxonomic institutes
Funding: EU

Project CPD 6.03.08: Mycoheritage. Reproduction of important classical illustration books on the Web
2005–2010: **J.A. Stalpers, G. Stegehuis, L. Reijers**

Project CPD 6.03.05: Digitalisation of collection data and Species Banks
2005–2006. **J.A. Stalpers, V. Robert, G. Stegehuis, P.W. Crous, R.A. Samson, G.S. de Hoog, L. Reijers, D. Stalpers, P. Meredith, S. Vanev,** NHN, ZMA, Naturalis
Funding: NWO

Scientific Output (2004–2005)

Scientific Publications

Alstrup V, Aptroot A (2005). Pyrenocarpous lichens from Kenya and Tanzania. *Cryptogamie Mycologie* **26**: 265–271.

Aptroot A (2004). Redisposition of some, mostly pyrenocarpous, lichen species described by Zahlbruckner from Taiwan. *Symbolae Botanicae Upsalienses* **34**: 31–38.

Aptroot A (2004). Two new ascomycetes with long gelatinous appendages collected from monocots in the tropics. *Studies in Mycology* **50**: 307–311.

Aptroot A, Berg MP (2004). *Collembola* help lichens in competition with algae. *Lichenologist* **36**: 167–169.

Aptroot A, Herk CM van (2004). *Caloplaca britannica* common in non-maritime environments. *Lichenologist* **36**: 261–263.

Aptroot A, Rodrigues AF (2005). New lichen records for the Azores, with the report of some tropical species new to Europe. *Cryptogamie Mycologie* **26**: 273–280.

Aptroot A, Zielman R (2004). *Lobaria amplissima* and other rare lichens and bryophytes on lava rock outcrop in the Eifel (Rheinland-Pfalz, Germany). *Herzogia* **17**: 87–93.

Ávila A, Groenewald JZ, Trapero A, Crous PW (2005). Characterisation and epitypification of *Pseudocercospora cladosporioides*, the causal organism of Cercospora leaf spot of olives. *Mycological Research* **109**: 881–888.

Ayala-Escobar V, Yañez-Morales M de J, Braun U, Groenewald JZ, Crous PW (2005). *Cercospora agavicola* – a new foliar pathogen of *Agave tequilana* var. *azul* from Mexico. *Mycotaxon* **93**: 115–121.

Ball LM, Bes MA, Theelen B, Boekhout T, Egeler RM, Kuijper EJ (2004). Significance of amplified fragment length polymorphism in the identification and epidemiology of *Candida* species colonization in children undergoing allogeneic stem cell transplantation. *Journal of Clinical Microbiology* **42**: 1673–1679.

Barnes I, Crous PW, Wingfield BD, Wingfield MJ (2004). Multigene phylogenies reveal that red band needle blight of *Pinus* is caused by two distinct species of *Dothistroma*, *D. septosporum* and *D. pini*. *Studies in Mycology* **50**: 551–565.

Barreto de Oliveira MT, Boekhout T, Theelen B, Hagen F, Baroni FC, Lazera M, Lengeler KB, Heitman J, Rivera ING, Paula CR (2004). *Cryptococcus neoformans* shows a remarkable genotypic diversity in Brazil. *Journal of Clinical Microbiolology* **42**: 1356–1359.

Batra R, Boekhout T, Guého E, Cabañes FJ, Dawson TL Jr, Gupta AK (2005). *Malassezia* Baillon, emerging clinical yeasts. *FEMS Yeast Research* **5:** 1101–1113.

Baumgardner DJ, Summerbell RC, Krajden S, Alexopoulou I, Agrawal B, Bergeson M, Fuksa M, Bemis C, Baumgardner MA (2005). Attempted isolation of *Blastomyces dermatitidis* from native shrews in northern Wisconsin, U.S.A. *Medical Mycology* **43**: 413–416.

Beilharz VC, Pascoe IG, Wingfield MJ, Tjahjono B, Crous PW (2004). *Passalora perplexa*, an important pleoanamorphic leaf blight pathogen of *Acacia crassicarpa* in Australia and Indonesia. *Studies in Mycology* **50**: 471–479.

Boer W De, Folman L, Summerbell RC, Boddy L (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29**: 795–811.

Boer W De, Leveau JHJ, Kowalchuk GA, Klein Gunnewiek WPJA, Abeln ECA, Figge MF, Sjollema K, Janse JD, Veen JA van (2004). *Collimonas fungivorans* gen. nov., sp. nov., a chitinolytic soil bacterium with the ability to grow on intact fungal hyphae. *International Journal of Systematic and Evolutionary Microbiology* **54**: 857–864.

Brandt ME, Kauffman CA, Pappas PG, Iqbal N, Arthington-Skaggs B, Lee-Yang W, Smith MTh (2004). Fungemia caused by *Zygoascus hellenicus* in an allogeneic stem cell transplant recipient. *Journal of Clinical Microbiology* **42**: 3363–3365

Braun U, Crous PW (2005). Additions and corrections to names published in *Cercospora* and *Passalora*. *Mycotaxon* **92**: 395–416.

Brodo IM, Aptroot A (2005). Corticolous species of *Protoparmelia* (lichenized Ascomycotina) in North America. *Canadian Journal of Botany* **83**: 1075–1081.

Bruyn U de, Aptroot A, Sparrius LB, Linders W (2005). Ergebnisse eines Flechten-Kartierungstreffens in Ostfriesland (Nordwest-Niedersachsen). *Aktuelle Lichenologische Mitteilungen NF* **14**: 18–29.

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Chitarra GS, Abee T, Rombouts FM, Dijksterhuis J (2005). 1-Octen-3-ol has mild effects on membrane permeability, respiration and intracellular pH, but blocks germination and changes the protein composition of *Penicillium paneum* conidia. *FEMS Microbiology Ecology* **54**: 67–75.

Chitarra GS, Abee T, Rombouts FM, Posthumus MA, Dijksterhuis J (2004). Germination of *Penicillium paneum* conidia is regulated by a volatile self-inhibitor. *Applied and Environmental Microbiology* **70**: 2823–2829.

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Coller GJ Van, Denman S, Groenewald JZ, Lamprecht SC, Crous PW (2005). Characterisation and pathogenicity of *Cylindrocladiella* spp. associated with root and cutting rot symptoms of grapevines in nurseries. *Australasian Plant Pathology* **34**: 489–498.

Crous PW, Allegrucci N, Arambarri AM, Cazau MC, Groenewald JZ, Wingfield MJ (2005). *Dematiocladium celtidis* gen. sp. nov. (*Nectriaceae*, *Hypocreales*), a new genus from *Celtis* leaf litter in Argentina. *Mycological Research* **109**: 833–840.

Crous PW, Gams W, Stalpers JA, Cannon PF, Kirk PM, David JC, Triebel (2004). An online database of names and descriptions as an alternative to registration. *Mycological*

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Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004). MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.

Crous PW, Groenewald JZ (2005). Hosts, species and genotypes: opinions versus data. *Australasian Plant Pathology* **34**: 463–470.

Crous PW, Groenewald JZ, Mansilla JP, Hunter GC, Wingfield MJ (2004). Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. *Studies in Mycology* **50**: 195–214.

Crous PW, Groenewald JZ, Pongpanich K, Himaman W, Arzanlou M, Wingfield MJ (2004). Cryptic speciation and host specificity among *Mycosphaerella* spp. occurring on Australian *Acacia* species grown as exotics in the tropics. *Studies in Mycology* **50**: 457–469.

Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hywel-Jones NL (2004). *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* **50**: 415–430.

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Denýzcý A, Kazan D, Abeln ECA, Erarslan A (2004). Newly isolated *Bacillus clausii* GMBAE 42: An alkaline protease producer capable to grow under highly alkaline conditions. *Applied Microbiology and Biotechnology* **96**: 320–327.

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Fisher MC, Hoog GS de, Johnson E, Smith M, White NJ, Vanittanakom N (2005). Clonal populations of *Penicillium marneffei* in Thailand reveal low effective dispersal. *PLOS Pathogens* **1**: 159–165.

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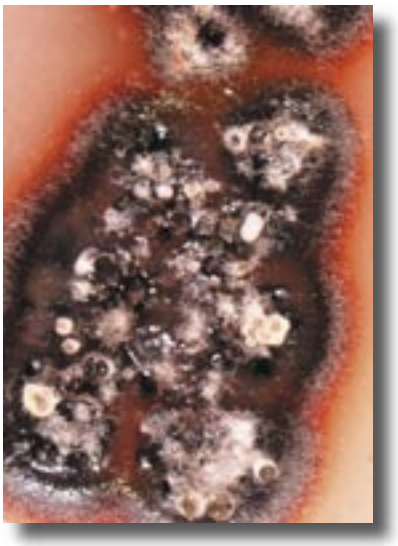

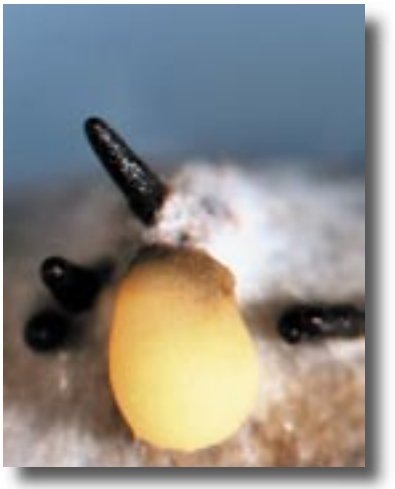

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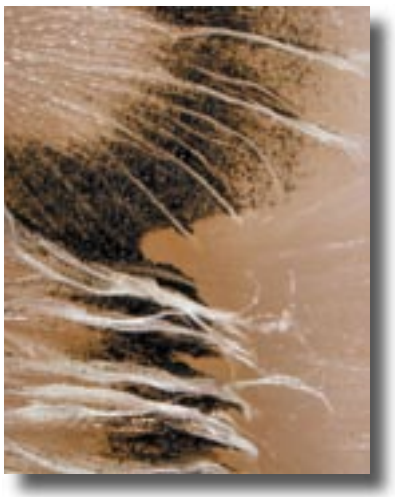

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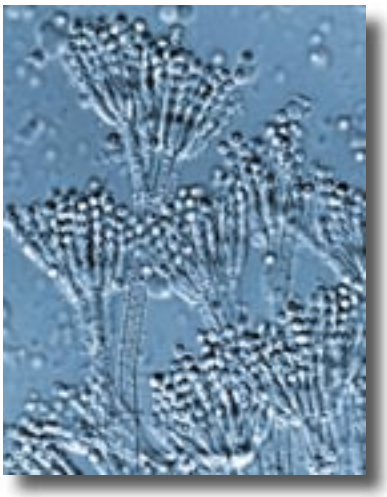

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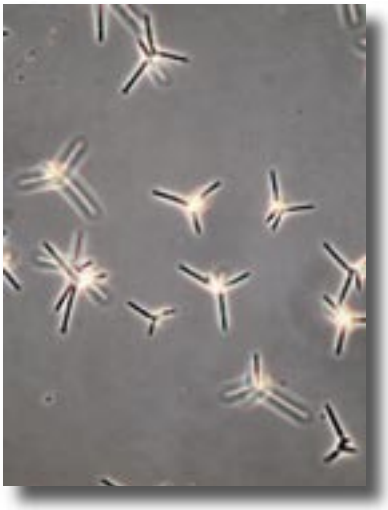

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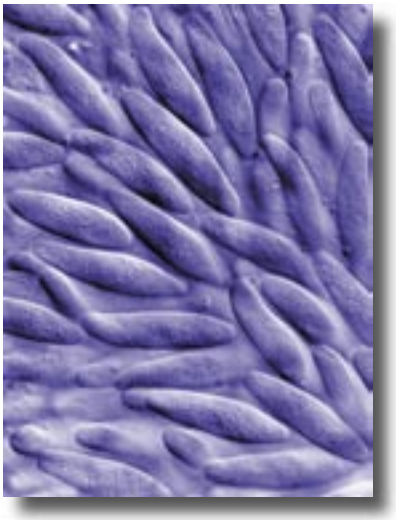

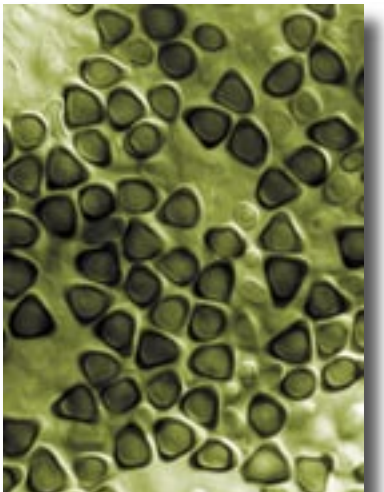

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Samson RA (2005). Current taxonomic concepts of species of *Aspergillus* and *Penicillium* producing important mycotoxins. 9th National Congress of Indonesian Society for Microbiology, August 24–27, Bali, Indonesia.

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Vitale RG, Schwarz P, Dannaoui E, Deng S, Machouart M, Kuijpers AFA, Hoog GS de (2005). Antifungal susceptibility and phylogeny of agents of zygomycosis. ICAAC, December 16–19 Washington, U.S.A.

Wright LP, Wingfield BD, Crous PW, Brenneman T, Wingfield MJ (2005). Developing polymorphic microsatellites for studying the population genetics of *Cylindrocladium parasiticum*. Annual meeting of the Southern African Society for Plant Pathology, 23–26 January, Western Cape, South Africa.

Zeng JS, Hoog GS de (2005). Diagnostics of black yeasts (*Exophiala* spp.) with the report of a novel, common clinical species, *E. xenobiotica*. November 29, Nederlandse Vereniging voor Medische Mycologie, Utrecht, The Netherlands.

Zeng JS, Sutton DA, Hoog GS de (2005).

Identification and pathogenicity of clinical isolates of genus *Exophiala* from the U.S.A. Section Medical Mycology of NVMM / NvMy, April 13, Arnhem, The Netherlands.

Other Scientific Activities

Staff served on the following societies, foundations, committees, etc.

Academy Colloquium in Fungal Phylogenomics, Amsterdam, May 11-12, 2004, Boekhout T (organiser).
Africa Fund for Fungal Biodiversity and Mycotic Infections, de Hoog GS (founder and member of board).
Australasian Plant Pathology, Crous PW (member of editorial board).
CBS Biodiversity Series, Crous PW, Gams W, Samson RA, Summerbell RC (members of editorial board).
Centre of Excellence in Tree Health Biotechnology, South Africa, Crous PW (member).
Centro de recursos microbiologicos, Universidade Nova de Lisboa, Boekhout T (member of scientific advisory board)
Christine Buisman Stichting, Crous PW (member of board).
Consortium of European Taxonomic Facilities, Crous PW (member of board).
ECMM Working Group Pseudallescheria Scedosporium Infections, de Hoog GS (co-ordinator).
ECCO meeting, Portugal, Stalpers JA (co-organizer & chair).
European Culture Collection's Organization (ECCO), Stalpers JA (member of board).
FEMS Yeast research, Boekhout T (adjunct editor in chief).
First Western European Workshop on Cryptococcus and cryptococcosis, Utrecht, The Netherlands, November 22, 2004, T. Boekhout (organiser).
Fungal Diversity, Aptroot A, Crous PW (members of editorial board).
GBIF (Global Biodiversity Information Facility), Stalpers JA (member of technical committee).
Gewasbeschermingsmiddelen stuurgroep, Crous PW (member).
International Commission on Food Mycology, Samson RA (treasurer)
International Commission on Indoor Fungi, Samson RA (chairman).
International Commission on Penicillium and Aspergillus, Samson RA (chairman).
International Commission for the Taxonomy of Fungi, Crous PW (member and co-ordinator of the *Mycosphaerella* subcommission), Samson RA (member).
International Mycological Association, Crous PW (member of executive committee).
International Mycological Congress (IMC8), Boekhout T (member of scientific committee)
International Society for Human and Animal Mycology (ISHAM), de Hoog GS (President-elect).
International Union of Microbiological Societies. Samson RA (Secretary General).
International Workshop on Esca and grapevine decline (ICGTD 4), *South Africa*, Crous PW (co-organiser).
IUMS – Mycology Division, Samson RA (chairman 2002-2005)
Johanna Westerdijk Stichting, Crous PW (member of board).
Journal of Plant Pathology, Crous PW (member of editorial board).
KREM (Dutch working group for Scanning Electron Microscopy), Dijksterhuis J (member of board).
Masterclass in Fungal Phylogenomics, Utrecht, May 10, 2004, Boekhout T (organiser).
Masterclass Fungal Ecology, Curitiba, Brazil, Nov. 16, 2005, de Hoog GS (organiser & lecturer).
Medical Mycology – The African Perspective, Hartenbosch, South Africa, Jan 25, 2005, de Hoog GS (organiser).
MSc Committee, *Van Coller GJ* (2004) University of Stellenbosch, Crous PW (co-supervisor).
MSc Committee, *Lubbe C* (2004) University of Stellenbosch, Crous PW (co-supervisor).
MSc Committee, *Pretorius MC* (2004) University of Stellenbosch, Crous PW (co-supervisor).
Mycological Progress, de Hoog GS (member of editorial board).
Mycological Society of America, Crous PW (member of culture collections committee).
Mycoses, de Hoog GS (managing editorial board).
Mycosphaerella leaf diseases of eucalypts, Australia, Geelong Crous PW (co-organiser).
National Research Foundation, South Africa, Boekhout T & Crous PW (peer reviews).
National Museum of Natural History Naturalis, Crous PW (member of scientific advisory board).
Natural Sciences and Engineering Research Council, Canada, Boekhout T & Crous PW (project reviews).
Netherlands Society for Medical Mycology, de Hoog GS (scientific secretary).
Netherlands Society for Microbiology, Boekhout T (chair of mycology section).
Netherlands Society for Microbiology, Boekhout T (member of board).
NL-BIF, the Dutch National Organisation Participating in GBIF (Global Biodiversity Information Facility), Stalpers JA (member of board).
NMV (Dutch Mycological Society), Stalpers JA (member of scientific committee).
NWO/ALW, Jury for Ecology, Biodiversity and Evolution Boekhout T (2003, member).
Nomenclature: Committee for Fungi, Gams W (secretary until Aug. 2005).
Nova Hedwigia, Gams W (mycology editor).
Odo van Vloten Stichting, Crous PW (member of board).
OECD (Organisation for Economic Co-operation & Development), Stalpers JA (Dutch representative on Biotechnology for Biological Research Centres).
Pan-African Medical Mycology Society (PAMMS), de Hoog GS (founder and co-organiser).
PhD Committee Ernst Jan Scholte EJ (2004) University of Wageningen. Samson RA (member of committee).
PhD Committee Ngo Thi Phuong Dung (2004) University of Wageningen. Samson RA (member of committee).
PhD Committee, *Van der Gaag M* (2005) University of Wageningen, Crous PW (member of committee).
PhD Committee, *Den Bakker HC* (2005) University of Leiden, Crous PW (member of committee).
PhD Committee, De Vos M (2005), University of Gent, de Hoog GS (member of committee).
PhD Committee, Hountondji FCC (2005), University of Amsterdam, de Hoog GS (member of committee).
PhD Committee, *Schoustra S* (2005) University of Wageningen, Crous PW (member of committee).

PhD Committee, *Schubert K* (2005) Martin-Luther University, Crous PW (member of committee).
PhD Committee, *Smith A* (2005) University of Tasmania, Crous PW (member of committee).
PhD Committee, *Hall T* (2004) University of New England, Boekhout T (member of committee).
PhD Committee, Te Dorsthorst (2005), University of Nijmegen, de Hoog GS (member of committee).
PhD Committee, *Phuong Dung NT* (2004), University of Wageningen, Boekhout T (member of committee).
PhD Committee, Wood A (2004) University of Stellenbosch, Crous PW (promoter).
PhD Committee, Halleen F (2005) University of Stellenbosch, Crous PW (promoter).
PhD Committee, Thanh NV (2004) University of Wageningen, Dijksterhuis J (member of committee).
PhD Committee, Rahardjo Y (2005) University of Wageningen, Dijksterhuis J (member of committee).
Proctor and Gamble, Cincinnatti, U.S.A., Boekhout T (consultant).
Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba, Japan, de Hoog GS (member of evaluation committee).
Research School of Biodiversity, Crous PW (member of board).
Section Mycology of the Netherlands Society for Microbiology, Boekhout T (chairman).
Studies in Mycology, Crous PW, Gams W, Samson RA, Summerbell RC (members of editorial board).
Systematic and Applied Microbiology, Samson RA (member of the editorial board).
University of Amsterdam, de Hoog GS (extraordinary professor).
University of Pretoria, Crous PW (extraordinary professor).
University of Stellenbosch, Crous PW (extraordinary professor).
University of Wageningen, Crous PW (extraordinary professor).
University Katsetsart Bangkok, Thailand, Samson RA (adjunct professor).
Willie Commelin Scholten Stichting, Crous PW (member of board).
World Federation of Culture Collections (WFCC), Stalpers JA (member of board).

CBS Seminar Series 2004

January 5: **Jos Houbraken**
Byssochlamys and its *Paecilomyces* anamorphs: taxonomy, food spoilage and mycotoxins

January 12: **Lizel Mostert**
Species delimitation in the genus *Phaeoacremonium*

January 19: **Sybren de Hoog, Bert Gerrits van den Ende, Guilliermo Fernández-Zeppenfeldt**
Possible pathogenicity of the *Cladophialophora carrionii* clade as inferred from phylogenetic comparisons

January 19: **G. Fernández-Zeppenfeldt**
Stenocereus cactus as the possible natural reservoir of *Cladophialophora* agents of chromoblastomycosis

January 26: **Teun Boekhout**
Cryptococcus neoformans: clinically relevant biodiversity

February 2: **Arthur de Cock**
Molecular phylogeny and the evolution of the 5S rRNA gene organization in *Pythium*

February 9: **Bart Theelen**
Use of microarrays in comparative mycology

February 16: **Ewald Groenewald**
Phylogeny of *Cercospora*: a Molecular Approach

February 23: **Jamal Harrak**
Black oligotrophic fungi in drinking water

March 1: **Marizeth Groenewald**
Molecular characterization of *Cercospora beticola*

March 8: **Gerard Verkley**
Annual cycle of an ascomycete community associated with oak leaves

March 15: **Shu-hui Tan**
Stability of freeze-dried fungi

March 22: **Vincent Robert**
Data integration and multi-factorial analysis, the yeasts as a case study

March 29: **Richard Summerbell**
How reliable is morphology in fungal identification and classification? The fusariologist's perspective

April 5: **Marjan Bovers**
Using *Caenorhabditis elegans* to study pathogenesis of *Cryptococcus neoformans*

April 5: **Ferry Hagen**
Epidemiology of *Cryptococcus neoformans* in The Netherlands. 25 Years of Cryptococcosis

April 19: **André Aptroot**
Revision of the genus *Amphisphaeria* and its implications for other groups

April 26: **Jan Dijksterhuis**
PLAY - An abundant ascospore cell wall protein

May 3: **Gerrit Stegehuis**
CBS databases, current developments

May 17: **Francesc Prenafeta**
Population and community level approaches for analysing interactions between fungi and bacteria in natural terrestrial environments

May 24: **Laura Selbmann, Sybren de Hoog and Silvano Onofri**
Fungi at the edge of life: crypto-endolithic black fungi from Antarctic deserts

May 24: **Sybren de Hoog & Joop van Brummelen**
Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica

June 7: **Maudy Smith**
Taxonomy of *Debaryomyces* Lodder & Kreger-van Rij

June 21: **Kenneth van Driel**
Characterization of the septal pore cap proteins of *Rhizoctonia solani*

August 23: **Eiko Kuramae**
Fungal Phylogenomics

August 30: **Joost Stalpers**
New Zealand, mycological and otherwise

September 6: **Edwin Abeln**
Setting up a cloning strategy for the mating type genes of *Mycosphaerella musicola* and *Mycosphaerella fijiensis*

September 20: **Robin May**

Cryptococcosis in *C. elegans* (or "When worms go yeasty…")

September 27: **Jos Houbraken**
Applied research: some industrial projects

October 4: **Lizel Mostert**
Pathogenicity testing of *Phialophora richardsiae*, *Phaeoacremonium*-like and *Acremonium* species on grapevines

October 11: **Bart Theelen**
Candida species with Real-Time PCR

October 18: **Mahdi Arzanlou**
Towards resolving the *Mycosphaerella* leaf spot complex of banana

October 25: **Ewald Groenewald**
Functional speciation in *Mycosphaerella*: Examples from the *Cercospora apii* complex

November 1: **Teun Boekhout**
The Vancouver Island outbreak of *Cryptococcus gattii*

November 8: **Marizeth Groenewald**
Genetic structure of *Cercospora beticola* populations

November 15: **Sybren de Hoog, Bert Gerrits van den Ende, Gé Poot & Maudy Smith**
Geotrichum: a fungal dynosaur?

November 22: **Gerard Verkley, Ewald Groenewald, Uwe Braun, Andre Aptroot and Pedro Crous**
Delimiting species in *Septoria* and in the *Ramularia*-clade of *Mycosphaerella*

November 29: **Shu-hui Tan**
The CBS Collection

December 6: **Vincent Robert**
How many genes do we need to sequence and which ones ?

December 13: **Victor Ursic**
Some fungi like it cold. *Aureobasidium* sp. in Arctic glacial ice

December 20: **Arthur de Cock**
Molecular detection of *Phytophthora* and *Pythium*

CBS Seminar Series 2005

January 10: **Francesc Prenafeta**
Fungi in bioremediation: Can the remedy be worse than the problem?

January 17: **Marjan Bovers**
Luminex *x*MAP™ TECHNOLOGY: a high-throughput detection and identification method

January 24: **Jan Dijksterhuis**
PLAY, a crucial factor in dormancy and heat-resistance of ascospores of *Talaromyces macrosporus?*

January 31: **Gerrit Stegehuis**
Integrating data: Index Fungorum, MycoBank, Biolomics, NWO

February 7: **Carlos Lopez**
Fungal diversity and litter decomposition in the National park Amacayacu, Dept. de Amazonas, Colombia

February 14: **Kenneth van Driel**
The Septal Pore Cap Structure: Enrichment and Characterization

February 21: **Ferry Hagen**
Vancouver Island *Cryptococcus gattii* outbreak

February 28: **Rob Samson**
Fungi in indoor environments

March 14: **Jamal Harrak**
Ecology of the genus *Cadophora*: plant endophytes?

March 21: **Hans-Josef Schroers** (Agricultural Institute of Slovenia) **& Richard Summerbell**
Taxonomy, phylogeny, phylogeography and patterns of opportunistic human pathogenicity within the *Fusarium dimerum* species complex

April 4: **André Aptroot**
Subfossil fungi

April 11: **Eiko Kuramae**
Gene content of pathogenic and non pathogenic *Saccharomyces cerevisae*: clues to pathogenicity?

April 18: **Joost Stalpers**
Deep Hypha, the Dictionary and the classification of the Basidiomycota

April 25: **Richard Summerbell**
Is *Simplicity* a genus?

May 23: **Lizel Mostert**
A polyphasic approach to *Phaeoacremonium* species identification

May 30: **Edwin Abeln**
Cloning the mating type genes of *Mycosphaerella fijiensis* and *Mycosphaerella musicola*

June 6: **Mahdi Arzanlou**
How complex is *Ramichloridium?*

June 13: **Teun Boekhout**
Functional diversity in *Cryptococcus neoformans*

June 20: **Ewald Groenewald**
Pitfalls of molecular phylogeny

June 27: **Marizeth Groenewald**
Distinct species exist within the *C. apii* morphotype

September 5: **Nina Zellerhoff** (RVTH - Univ. of Aachen, Germany)
Host interactions with *Magnoporthe grisea*

September 12: **Gerard Verkley**
Phenotypic characterization of *Septoria* spp.

September 19: **Richard van Leeuwen**
Natamycin as a perturbator of the fungal membrane

September 26: **Mark van Passel** (Academic Medical Center, University of Amsterdam)
Compositional comparisons of chromosomes based on the genome signature

October 3: **Jan Dijksterhuis**
Differentiation inside multicelled macroconidia of *Fusarium culmorum*during early germination

October 10: **Gerrit Stegehuis**
Species banks

October 17: **Kenneth van Driel**
Enrichment of Septal Pore Caps in *Rhizoctonia solani*: Identification of SPC18, a putative SPC protein

October 24: **Rob Samson**
Studies in *Aspergillus*

October 31: **Marjan Bovers**
Unique hybrids between fungal pathogens

Cryptococcus neoformans and *Cryptococcus gattii*

November 7: **Montarop Sudhadham**
Genetic diversity of the neurotropic black yeast *Exophiala dermatitidis* and its association with wild animals

November 21: **Javier Cabañes** (Veterinary Mycology Group, Autonomous University of Barcelona)
Notes on *Malassezia* spp. from domestic animals

November 28: **Jingsi Zeng**
Diagnostics of black yeasts (*Exophiala* spp.) with the report of a common novel species, *E. xenobiotica*

December 5: **Bart Theelen**
Cryptococcus MicroArray Research: Current stage

December 12: **Wouter Los** (University of Amsterdam)
Towards the European Distributed Institute of Taxonomy (EDIT) DNA barcoding, and the role of the CBS

December 19: **Tino Ruibal** (Merck, Madrid, Spain)
The emergence of a highly successful extremotolerant clade of melanized fungi

CBS Special seminars 2005

June 8: **Constantino Ruibal** (Merck, Madrid, Spain)
Isolation and characterization of melanized, slow-growing fungi from semiarid rock surfaces of central Spain and Mallorca

September 15: **Wolfgang Albrecht** (Bruker Daltonics, GMBH, Bremen)
Rapid identification of Micro-Organisms using MALDI- TOF

Contract Research and Services

Food and Feed Mycology

***Heat-treated products*:** Samples of pectin (47 samples), canned strawberries (12 samples) and pasteurised fruit yoghurt were received for analyses aimed at detecting heat-resistant fungi. In many cases no heat-resistant fungi could be detected in pectin; occasionally, *Talaromyces trachyspermus* was isolated. This fungus, together with *T. assiutensis,* was the most prominent spoilage organism associated with the canned strawberries. Research is currently being conducted on the effect of heat treatments on the survival rate of *T. trachyspermus*.

Air sampling in bakery.

***Vegetables and fruits*:** In a few cases, the CBS was consulted for analysis of spoiled fruits. Samples of different types of berries and pears were investigated. The interest of the sender lay mainly in the possible presence of mycotoxigenic fungi. The data obtained were used for the improvement of the business's Hazard Analysis and Critical Control Point (HACCP) plan. *Botrytis* and yeasts were commonly isolated from the berry samples, while *Fusarium avenaceum* and *Cladosporium herbarum* were present in the pear samples.

***Bakery products*:** Various bakery products, including rye bread, donuts, bagels, tortillas, cookies (containing almond paste) were investigated for the presence of fungi, as were a diversity of ingredients for bakery products. As anticipated, members of the osmotolerant genus *Eurotium* were often detected; interestingly, the chalk fungus, *Endomyces fibuliger,* was also frequently encountered. Almond paste, which is used in some type of cookies, is not heat treated (baked) during the production process, and is therefore a potential source of contamination. The initial contamination level of various almond paste ingredients, e.g., milled rice, almonds and soybeans, was determined. High counts of *Eurotium* were detected in the milled rice.

***Dairy products*:** Many different dairy products, like yoghurt, butter, mozzarella cheese, chocolate milk, smoothies and dried milk powder were investigated. Related environmental samples were also frequently analysed. Strikingly, *Penicillium camemberti* was frequently present on portion-packaged cheese. *P. camemberti* is the domesticated form of *P. commune* and is generally only found in the production area of white mould cheeses (brie, camembert etc.). Since this species essentially never grows outside the white mould production environment, contamination of these packaged cheeses had clearly occurred inside the production plant, specifically, in its refrigerators.

Small green spots of *P. roqueforti* were detected on vacuum-packaged cheese. These spots had already formed before packaging took place. Since *P. roqueforti* is capable of surviving at low oxygen levels, the colonies seen were not dead but rather dormant, ready to form distinct colonies on the vacuum-packed cheese when sufficient oxygen again became available.

Eurotium *spoilage of bread.*

***Feed*:** Occasionally feed samples were received. Silage samples were investigated for the presence of actinomycetes and fungi, and grass samples were screened for the presence of *Pithomyces chartarum* conidia. Microscopic examination of the grass samples showed high numbers of *Pithomyces* conidia present. The cattle eating this grass showed symptoms of pithomycotoxicosis.

Collecting samples in a food factory.

***Fungi in indoor environments*:** Numerous samples of building materials such as wallpaper and plaster were examined, as well as wall scrapings. In addition, swab samples and cellotape impression samples from indoor surfaces were received. Samples came from museums, archives, private dwellings and schools. About 20 wood samples were analysed for the presence of wood rot fungi. Species belonging to the genera *Antrodia*, *Gloeophyllum*, *Oxyporus*, *Trametes*, *Meripilus* and *Phellinus* were detected; however, the extremely damaging dry rot fungus *Serpula lacrymans* was not detected.

Surveys, audits and inspection reports

***Surveys*:** In 2004 and 2005 several on-site investigations were performed in indoor environments, including nine archives and eight private dwelling. Besides air and surface sampling, materials and objects from these environments were investigated. In the archives, *Aspergillus penicillioides* was often the prevalent organism. Private dwellings were inspected, often with the goal of detecting possible allergenic fungi growing in these indoor environments. In three of the nine dwellings investigated, *Stachybotrys chartarum* was detected. This species is known to form highly cytotoxic macrocyclic trichothecenes and to interact with pulmonary cellular immune system components in a way that might explain some of the symptoms experienced by people in heavily *Stachybotrys*-contaminated dwellings. In some dwellings high numbers of fungal particles could be detected in air samples. Sensitized individuals, particularly asthmatic individuals, might experience strong allergic reactions in contact with these high fungal allergen levels.

Several bakeries producing rye bread, cakes or modified-air-packaged baguettes (French bread loaves packed in a low-oxygen gas mixture) were surveyed. In case of the modified-air-packaged baguettes, at the end of the shelf life, green and brown spots could be observed on the surface. The green spots were mostly formed by *Penicillium commune*, while the brown spots were formed by bacteria in the genus *Bacillus*. Thermal death curves were made for these *Bacillus* species and the data from these experiments were compared to those for the heat levels attained in the production process. The results confirmed that the baking process was not sufficiently hot to eliminate the initial *Bacillus* contamination.

In different bakeries producing rye bread, extended surveys were carried out. Besides air sampling, swabs were done and material samples were also collected. *Penicillium roqueforti* and *P. paneum*, both preservative-resistant species, were frequently encountered in these factories. In some cases, they were also encountered after the baking process.

Fusarium oxysporum and *F. solani* were frequently detected in heat-treated drinks. Surveys in the production and container filling plants were conducted. They showed that the fungi often occurred in high numbers in and around the filling machines. Further research was done to establish the route by which contamination with these fungi became established at those sites.

***Audits and expert reports*:** Expert reports and audits were made for various companies. We were consulted in connection with tempeh production (an Indonesian fungal fermentation process for soy), as well as with questions about *Beauveria bassiana*, second opinions on experiments done by other companies, legal business cases and audits of production plants (e.g., for improving HACCP plans).

Applied research

Experiments were preformed on the survival of *Zygosaccharomyces baillii* in samples containing alcohol. The effect of the alcohol percentage and the contact time with the alcohol was tested. Many experiments were preformed to establish the effect of preservatives on tulip bulbs. Tulip bulb rot is often caused by *Fusarium oxysporum*, *Penicillium hirsutum*, *P. tulipae* or *Aspergillus niger*. Both laboratory and field tests were conducted.

Genetically engineered bacteria

The Netherlands Ministry of Housing, Spatial Planning and the Environment has made CBS responsible for routine testing of genetically engineered micro-organisms (GMO's). A total of 68 GMO's were analysed. Most of these GMO's belonged to *Escherichia coli* but also samples from other bacterial species like *Campylobacter jejuni, Lactococcus lactis, Leuconostoc mesenteroides, Salmonella typhimurium* and yeasts such as *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* were analysed. As part of these tests phenotypic characterisation, partial16SrDNA, partial26rDNA and plasmid DNA restriction analysis were performed.

Mycotoxin analyses

Fungal strains used for the industrial production of enzymes or acids should not produce mycotoxins. In this context, fungal production strains (mainly *Aspergillus niger* and *A. oryzae*) were screened for toxin production. Also fermentation broths and concentrates made using these strains were investigated. This research was performed in collaboration with Biocentrum, Danish Technical University, Lyngby, Denmark.

Miscellaneous

The CBS was also consulted for mould problems in many other types of samples, e.g. medicines, potting soil, leather, plants and tattoo paint. A survey was conducted in a

leather production plant, specifically to detect the fungus *Hormoconis resinae*. This fungus was the main causal agent of brown spoting that made leather supplies useless for shoe manufacture. Research showed that the fungus was highly resistant to the fungicides used during the production.

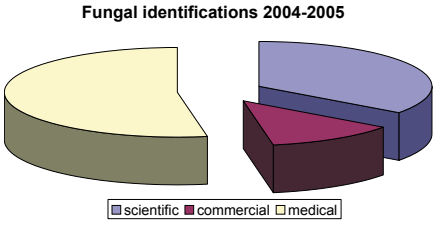
In the years 2004 and 2005 the CBS, in collaboration with the Dutch Food and Consumer Product Safety Authority, investigated samples of tattoo paint for bacteria and moulds. High numbers of moulds were detected, predominantly consisting of *Fusarium solani*, *Aspergillus sydowii* and *Scopulariopsis brevicaulis*.

Bacterial identification service

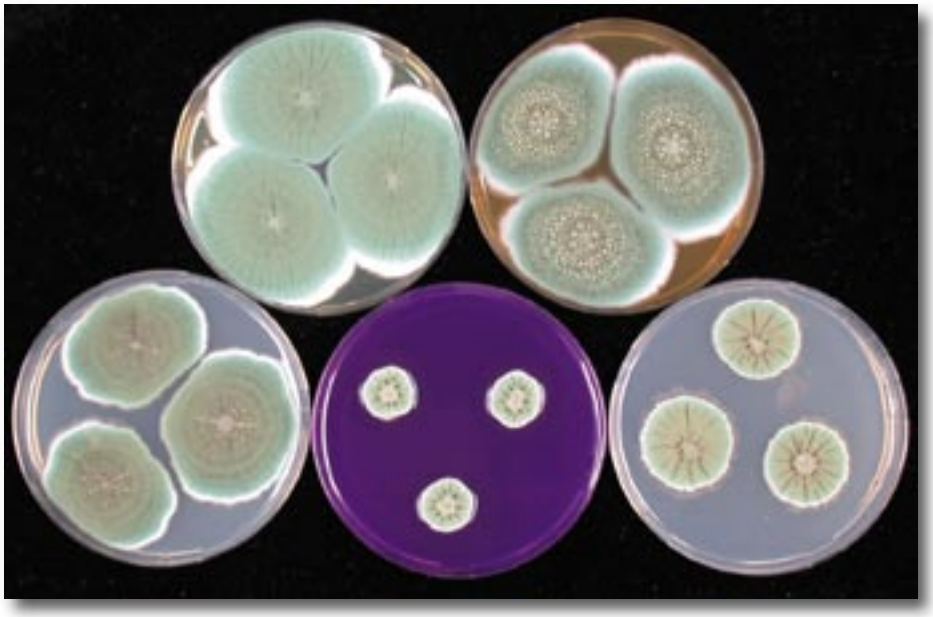
A total of 137 samples were identified for external customers by means of partial 16S rDNA analysis, as well as phenotypic characterisation in commercial systems such as API or Biolog. Samples like chocolate milk, soja souce, bread, flour, onions, biological pesticide and coolant were received for isolation and identification of bacteria. Additional services included the freeze-drying of strains (345 ampoules in total) and characterisation of *Escherichia coli* mutants.

Fungal identification service

CBS offers a service for the identification of fungal and oomycetous isolates. It employs experts for all major groups of fungi. The knowledge of these staff members guarantees a state-of-the-art identification of cultures. A correct identification is of the utmost importance both in scientific studies, phytopathology, industrial contamination etc.



As a consequence of developments in the field of taxonomy, a reorganization of the identification service was initiated in 2005. Application of molecular methods in particular is now often required for a proper identification. Yeasts are now identified mainly by means of DNA sequencing. A typical identification of a filamentous fungal isolate still starts with a morphological study on the appropriate media under the appropriate conditions. If a reliable morphological identification is not possible because structures seen are nondiagnostic or isolates are sterile, physiological and molecular methods will be applied to obtain the best possible identification. CBS has a significant unpublished database of DNA sequences, based on and ex-type and other well-characterised CBS strains and this information can be used by CBS experts to arrive ultimately at a reliable identification. This approach has resulted in identification to the species level in almost every case. In the few cases where the isolates remained sterile, or where no molecular database is available, the phylogenetic relationships of the isolates could be determined. A secondary effect of the revised procedures is that the identification service is able to inform a significant minority of clients that the isolate they have sent in represents a new species. It is now far easier to fully confirm species as undescribed that it was in the past, when only morphological characters were available. In many cases, approval of the customers was obtained to add the undescribed species and many other interesting isolates to the CBS collection. The identification service yielded 89 highly interesting isolates for the collection in 2004, and 100 isolates in 2005.



Penicillium *colonies on different media for phenotypical identifcation.*

An analysis in 2005 showed that prices charged by CBS for identifications were far below the actual costs. The prices have now been raised to a more realistic level, starting from January 1, 2006.

Fungal courses

• Fungal Biodiversity Course: an introduction. This course was held in 2004, but not in 2005. Participants in recent years have come from countries such as Sweden, Germany, the Netherlands, Belgium, Denmark, Italy, Thailand, Turkey, Indonesia, the USA, Iran, Estonia, Finland and Poland. This course is currently under revision for 2006.

• Medical Mycology course (2004 and 2005). This course was attended by more than 75 participants from all over the world.

• Introduction to Food-and Airborne Fungi Course (2004 and 2005). This course was attended by participants from China, Germany, Italy, Hungary, Sweden, Belgium, and the Netherlands. The course was also given in 2004 and 2005 in Ottawa in collaboration with Agriculture Canada, and in Bangkok in 2005 in partnership with the National Centre for Genetic Engineering and Biotechnology (BIOTEC).

A three-day course intended for industrial hygienists and others working in indoor environments (hospitals, building industries, analytical labs, etc.) was given in Stuttgart in collaboration with the Landesgesundheitsamt. Other one-day practical mycology courses were given in Lübeck, Germany in 2004 and 2005.

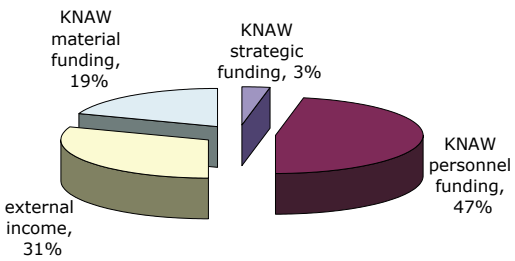
Finances and Staff

Income

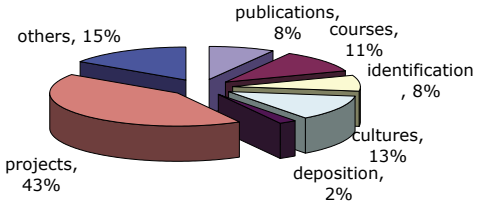
The Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre (CBS-KNAW) has a total income of 4.1 million Euros (Fig. 1). Approximately 69 % of this amount is KNAW funding. A further subsidy recently obtained from the KNAW strategic-fund is destined to be used for initiating innovative research projects. CBS has chosen to establish a DNA bank (NL-Bank), and to strengthen its DNA Barcoding projects by appointing a third post-doc to barcode the type strains of the CBS collection.

The external income (Fig. 2) of 1.3 million euros is profit earned mainly from research projects and regular activities, such as the sale of books, courses and the training of scientists and students, identification/sales of fungi and bacteria. The “Odo van Vloten” Foundation currently finances four Ph.D. research projects.

income 2005



external income

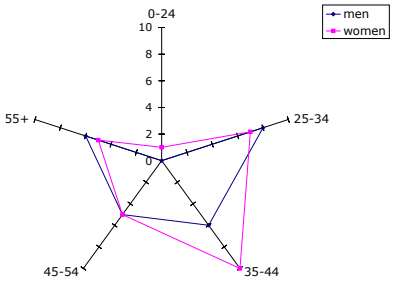


Expenditures

The total costs of the CBS-KNAW consist mainly of salaries (70 %). Non-personnel costs are costs of materials and depreciation of durable equipment.

Three-quarters of the expenditures (Fig. 3) can be indicated as direct research costs. Indirect costs are for management and for the collective supporting division of the CBS-KNAW and its neighbouring institute, the Hubrecht Laboratory (HL).

CBS staff divided by gender

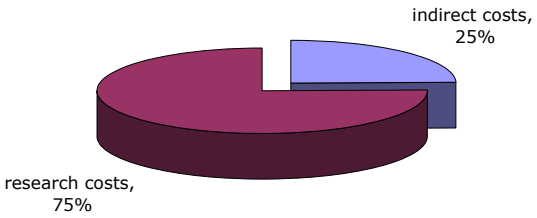


Staff

The CBS had 53 employees on January 1 2006, with an equivalent of 46,1 full-time staff (fte). The staff consists of 23 researchers and 30 analitical/technical support staff. A considerable proportion of the support staff is involved in the applied research division, fungal preservation, and digitalisation of data pertaining to the collection. Approximately 20 additional persons, guest researchers, researchers with an official appointment other than the CBS and students have been working at the CBS. Within CBS 53 % of the employees are female, and 47 % male (Fig. 4).

The collective support division CBS/HL employs 27 people with a full-time equivalent of 24,1 fte, with approximately 7,8 fte effectively working for the CBS.

expenditures 2005



CBS staff (2004–2005)

Directorate

Prof. dr P.W. Crous	Scientific Director
J. Koelman	Deputy Director Management
M.J. van den Hoeven-Verweij	Management Assistant

The Collection

Dr J.A.J.M. Stalpers	Curator
C.S. Tan	Deputy curator
Dr V.A.R.G. Robert	Deputy curator
Dr E.C.A. Abeln	Deputy curator
G.J. Stegehuis	Technician
B.P.M. Merkx	Technician
W.W.M. Epping	Technician
W. Haisma	Technician
J. Holtman	Technician
C.W. Jong-de Vogel	Technician
E. Mul	Technician
A.B.E. de Nooijer	Technician
M. Setropawiro	Technician
J. Snippe	Technician
F.B. Snippe-Claus	Technician
C.J. Verwoerd-Kuyt	Technician
IJ.A. Vlug	Technician
D. Vos-Kleyn	Technician
M.J. Figge	Technician
J.H.C. Woudenberg	Technician
A.T. Lugtenburg	Technician
L.A.M. Reijers	Administrative Assistant
T.M.A. Stalpers-den Brinker	Administrative Assistant
Dr. D. Yarrow	Guest researcher

Comparative Genomics and Bioinformatics

Dr T. Boekhout	Programme Leader
Dr E.E. Kuramae	Post-doc
K.G.A. van Driel	PhD student
M. Bovers	PhD student
F. Hagen	Technician
B.J.F. Theelen	Technician
G. Dingemans	Technician

Biodiversity & ecology

Dr R.C. Summerbell	Programme Leader
Dr A. Aptroot	Scientist
Dr G.J.M. Verkleij	Scientist
Dr M. Smith	Scientist
Dr F.X. Prenafeta-Boldu	Post-doc
C. Lopez Quintero	PhD student
M. Silvestri	PhD student
I.M. van Kempen	Technician
A.L. van Iperen	Technician
G.A. Poot	Technician
M. Starink-Willemse	Technician
Prof. Dr. K.W. Gams	Guest researcher
Dr H.A. van der Aa	Guest researcher

Indoor Air, Food and Applied Mycology

Prof. dr R.A. Samson	Programme Leader
Dr J. Dijksterhuis	Scientist
E.H. Hoekstra	Scientist
J.A.M.P. Houbraken	Technician
A.F.A. Kuijpers	Technician
C.C. van den Tweel-Vermeulen	Technician
M.J. Pouw	Technician
E. Dekker	Technician
C.J. van den Berg-Visser	Secretary
Y. Stoop	Student

Evolutionary Phytopathology

Prof. dr P.W. Crous	Programme Leader
Dr A.W.A.M. de Cock	Scientist
Dr J.Z. Groenewald	Post-doc
Dr H.J. Schroers	Post-doc
M. Groenewald	PhD student
L. Mostert	PhD student
M.M. Aveskamp	
M. Arzanlou	PhD student

Origins of Pathogenicity in Clinical Fungi

Prof. dr G.S. de Hoog	Programme Leader
M. Sudhadham	PhD student
M.J. Harrak	PhD student
A.H.G. Gerrits v.d. Ende	Technician
K.F. Luijsterburg	Technician

Library

M.T. Vermaas	Librarian

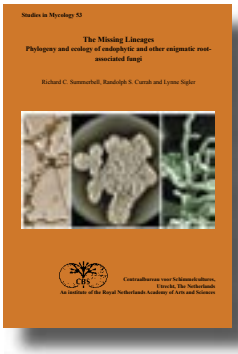
Support services

E.C.A. Freund
J.C.M. de Bruin
B.H.H. de Deugd
A.S.M.M. Maas
J. Norbruis
S.K. Raghoebir
D.A.J. van Velzen
I. Versluis
R. Davids
J.L. Deel
M.E. van Domselaar
R. van Voorst
J.H. Beeker
H.R. Reitsma
J.A. Seco Rodriguez
R.S. Verboekend
W.N.M. Geers
J. Heinen
H.L. Krielen
G. van de Lagemaat
R.H.T. van Pinxteren
P.J.M. van Arum-Swanink
A.L. van den Breul
R.C. Vermeulen
R. van der Waals

CBS Publications 2004–2005

Studies in Mycology:

Studies in Mycology is an international journal that publishes systematic monographs of filamentous fungi and yeasts, and on occasion the proceedings of special meetings related to all fields of mycology, biotechnology, ecology, molecular biology, pathology and systematics. Since 2004, it has been an open-access journal that is freely available on the internet, though the hard copy version is still available reasonably priced. The journal now has a full colour format, and is directly linked to MycoBank, with all papers linked to strains in the CBS collection that are available to the international scientific community. (http://www.cbs.knaw.nl/simonline/).



SIM 53: The Missing Lineages: Phylogeny and ecology of endophytic and other enigmatic root-associated fungi - Richard C. Summerbell, Randolph S. Currah & Lynne Sigler (editors): 254 pp., 2005.

SIM 52: Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (*Ascomycota*, *Diaporthales*, *Valsaceae*) from *Eucalyptus* - Gerard C. Adams, Michael J. Wingfield, Ralph Common and Jolanda Roux 146 pp., 2005.

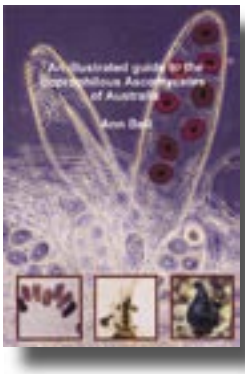
SIM 51: Fungi of the Antarctic: Evolution under Extreme Conditions - G. Sybren de Hoog (editor): 82 pp., 2005.

SIM 50: CBS Centenary: 100 Years of Fungal Biodiversity and Ecology(Two parts) - Pedro W. Crous, Robert A. Samson, Walter Gams, Richard C. Summerbell, Teun Boekhout, G. Sybren de Hoog and Joost A. Stalpers: 580 pp.

SIM 49: *Penicillium subgenus Penicillium*: new taxonomic schemes, mycotoxins and other extrolites - Robert A. Samson and Jens C. Frisvad: 2004.

CBS Biodiversity Series:

The CBS Biodiversity Series is an international publication on filamentous fungi and yeasts and publishes systematic monographs related to all fields of mycology including biotechnology, ecology, molecular biology, pathology and systematics.



CBS Biodiversity Series 3: An illustrated guide to the coprophilous *Ascomycetes* of Australia - Ann Bell: 173 pp, 115 plates (A 4 format), paperback with spiral binding, 2005

CBS Biodiversity Series 2: Cultivation and Diseases of *Proteaceae*: *Leucadendron*, *Leucospermum* and *Protea* - Pedro W. Crous, Sandra Denman, Joanne E. Taylor, Lizeth Swart and Mary E. Palm: 571 pp.

Books and CD roms:

Introduction to Food - and Airborne Fungi (Robert A. Samson *et al.*), seventh edition. 389 pp., 2004.

Atlas of Clinical Fungi CD-ROM (G Sybren de Hoog *et al.*), 2005.

Popular Scientific Activities

The CBS website has attracted much attention. In 2004-2005 the site has been visited over 8.500.000 times with an average of 24.000 per day. Many visitors consult the CBS collection and other databases, while the PDF's of the Studies in Mycology are often downloaded.

In 2004 CBS celebrated its centenary with a symposium and several festivities. This anniversary received much attention in the Dutch newspapers, television and radio programmes.


Cultures Oudste bewaarplaats voor schimmels viert honderdjarig bestaan

CBS viert 100-jarig bestaan

Geen leven zonder schimmels

Het leuke van schimmels

Grootste collectie ter wereld

Bejaarde kwekelingen zijn nog fit

Children learn about fungi
CBS contributed to the programme "Nieuws uit de Natuur" on Dutch national television in November. The programme is part of an educational series on biology designed for children in elementary school. Programme presenter Mylene learned more about culturing fungi in the CBS labs, and about mushrooms in the field under the guidance of CBS's Gerard Verkley, whom she "unexpectedly" bumped into on her foray in a nearby forest. Here Gerard is explaining the principle of gravitropism in basidiocarps of *Piptoporus betulinus*.