

Mycology: unravelling the riddle of the filamentous fungi

The fungi are perhaps the least understood of the multicellular organisms, despite being almost ubiquitous in nature. An international team, coordinated by **Professor Dr Reinhard Fischer** of the Karlsruhe Institute of Technology, Germany, and **Professor Dr Meritxell Riquelme** from the Centro de Investigación Científica y de Educación Superior de Ensenada, Mexico, is leading attempts to understand the growth and development of these remarkable organisms, shedding light on their medical and ecological applications.

There may be as many as five million species of fungi worldwide – many more than there are plants. The vast majority of these little-understood organisms are the ‘filamentous fungi,’ named because they are composed of a web of filaments called ‘hyphae’. The work of Professor Fischer, Professor Riquelme and their co-workers focuses on how these filaments grow, indefinitely, by

extension at each microscopic tip, to form huge networks called ‘mycelia’.

UNDERRATED ORGANISMS

Despite their lowly appearance, the filamentous fungi are crucial to the functioning of natural ecosystems. Alongside bacteria, they are the main agents responsible for decomposing dead organic matter, making its chemical components available to the next generation of organisms. What is perhaps

Left: The picture shows different colour mutants of *Aspergillus nidulans*. Wild type forms green spores which cover almost the entire colony. It is easy to generate mutants in which pigment biosynthesis is blocked at certain stages and thus a different pigment variant (yellow) or no pigments (white) are produced. Mutants are a great starting point for a molecular analysis. This approach has been used to study many cellular processes and made *A. nidulans*, a model organism for lower eukaryotes and beyond. The diameter of a colony is about 1 cm.

less well-known is that they play a vital role, not only in generating nutrients, but also in plant nutrient uptake: every metre of plant root in the soil is associated with roughly a kilometre of symbiotic fungal hyphae, known as ‘mycorrhiza’, which take up nutrients and pass them to the plant.

Filamentous fungi are important pathogens of crop plants, and in a few cases cause serious human disease, particularly in the immunocompromised. They have also been harnessed for biotechnological uses, including crucially in the production of antibiotics such as penicillin, other medicines, citric acid, and foods such as soy sauce and cheese. To scientists, fungi are also important due to the similarity of their cells to human cells, making them ideal models to study various aspects of cell function.

Professor Fischer, Professor Riquelme and their colleagues, with funding from the Deutsche Forschungsgemeinschaft and CONACYT, are studying a host of questions surrounding the growth and development of filamentous fungi. Using multiple species and state-of-the-art microscopy and molecular biological methods, they are enhancing our understanding of the mechanisms by which these intriguing and important organisms grow and differentiate.

KEEPING PACE WITH GROWTH

At the tip of each fungal hypha lies a region of active growth. Here, membrane-bound particles (vesicles) containing the raw materials for building new cell walls and membranes – proteins, lipids and other organic molecules, as well as catalytic enzymes – fuse with the cell’s boundary membrane, releasing their precious cargo. However, the highly polarised positioning of this region poses challenges for the fungus. Firstly, how can they transport

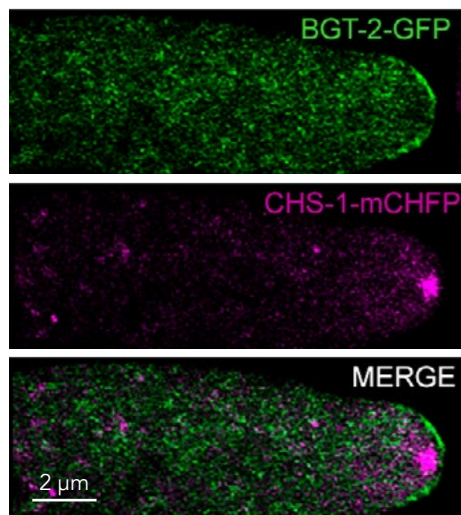


Above: With the help of the jellyfish green fluorescent protein (GFP) researchers visualised the microtubule cytoskeleton in *Aspergillus nidulans*. Round spores produced long hyphae, and microtubules are visible as long filaments in the cells. They serve as tracks for intracellular traffic. Picture taken by Minoas Evangelinos, KIT.

adequate quantities of these materials to the tip in order to keep up with the rate of growth? Secondly, with such rapid growth occurring at the tip, how does the hypha maintain a stable marker of exactly where, and in what direction, growth is to occur?

The logistics of transporting materials to the actively-growing hyphal tip are being elucidated by Michael Feldbrügge’s lab at Heinrich Heine University, Düsseldorf, Germany. The length of the hyphae is traversed by a skeleton of fine tubes called microtubules, along which vesicles and their contents travel, facilitated by proteins acting as motors. Feldbrügge has also found that these provide transport routes for molecules of messenger RNA, which translate the genetic information in DNA into functional proteins. Crucially, this means that protein production can be precisely targeted to specific regions within a cell without having to transport large quantities of the proteins themselves.

The diverse and enlightening findings of this high-profile programme have implications far beyond the fungal



Above: In this experiment two enzymes required for cell wall synthesis were visualised in hyphae of *Neurospora crassa* using two different coloured fluorescent proteins. Whereas an endoglucanase enzyme (BGT-2) localises to the plasma membrane, a chitin synthase (CHS-1) accumulates first in a structure called "Spitzenkörper" before it is secreted. Confocal Laser scanning microscopy images obtained by Dr Leonora Martínez-Núñez, CICESE.

In answer to the second question, Prof Fischer himself, working with Prof Norio Takeshita, has discovered that in the filamentous fungus, *Aspergillus nidulans*, molecules of a special protein – TeaR – located at the tip of the hyphae, mark the zone in which active growth is taking place. Using advanced, super-resolution microscopy techniques to visualise the activities of living cells in real time, they showed that the cluster of TeaR molecules at the hyphal tip is repeatedly dispersed and reassembled with newly-arriving TeaRs, maintaining an indicator that tells the machinery of the cell where to build new tissue.

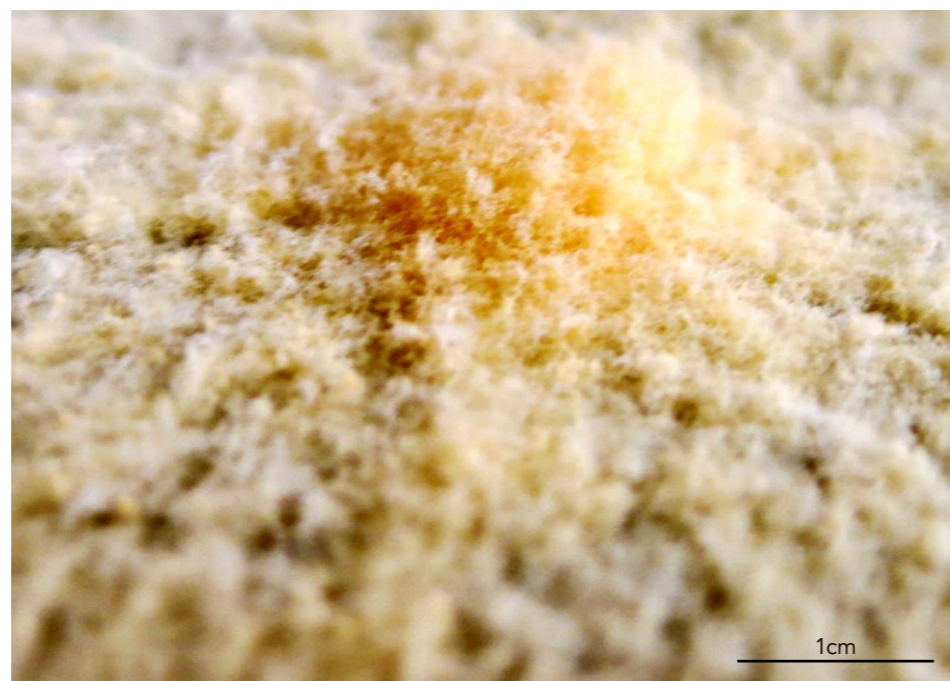
A further project, led by Prof Meritxell Riquelme is shedding light on the precise nature of the transport of vesicles found at the growing hyphal tip. Remarkably, it turns out that there are separate populations of differently-sized vesicles carrying different enzymes for building various components of the fungal cell wall.

FROM REPAIR TO REPROGRAMMING

In addition to spontaneous growth, organisms also need to repair themselves from damage, and research into filamentous fungi is shedding light onto how this may be achieved. Prof Alfredo Herrera-Estrella at the Laboratorio Nacional de Genómica para la Biodiversidad, Guanajuato, Mexico, and colleagues, use the fungus *Trichoderma atroviride*, a biocontrol agent, in their work. They have shown, using modern genetic approaches,



Close-up images of a colony of *N. crassa*. The aerial mycelium shows the interwoven fine hyphae. Carotene stains the mycelium orange. Pictures taken by Rosa Aurelia Fajardo Somera from KIT.



State-of-the-art microscopy and molecular biological methods are enhancing our understanding of the mechanisms by which these intriguing and important organisms grow and differentiate

Q&A

How numerous, widespread and significant are the filamentous fungi?

Fungi are found in nearly all ecosystems, where they fulfil important functions for nutrient recycling. Some species are important plant pathogens, such as *Magnaporthe oryzae* or rust fungi. There are also animal pathogens. Many moulds contaminate food and feed and cause tremendous losses due to mycotoxin formation.

Why is their growth and development such an interesting area to study?

The fungal hypha is able to grow indefinitely at the tip. It is one of the few examples of extreme polar growth of individual cells. Other examples are pollen tubes, root hairs and nerve cells. Thus the study of the mechanisms of polar fungal extension may help to improve our understanding of polarity in general. Likewise, simple hyphae are able to differentiate rather complicated structures such as fruiting bodies. This requires massive changes in gene expression. It can be an example for other differentiation processes, e.g., embryogenesis in higher eukaryotes.

that injury results in the production of highly reactive and damaging molecules known as 'reactive oxygen species (ROS)', stimulating the formation of reproductive structures. This molecular pathway promotes cell differentiation and regeneration in the face of damage and – using ROS as signals – could be shared with both plants and animals. Therefore, understanding it could have important applications in medicine.

Also working at the Karlsruhe Institute, Prof Natalia Requena is studying how filamentous fungi interact with plants when forming arbuscular mycorrhizal symbioses, which improve the plants' supply of phosphate, sulphur and nitrogen in return for carbohydrates. Within roots, the fungi form specialised structures known as 'arbuscules' to allow the nutrient exchange between symbiotic partners. Arbuscule

What recent technological advances have helped further your research?

The advent of molecular biological methods in the 1980s, the use of GFP and other fluorescent proteins since 1994 in combination with steadily improved microscopy techniques and the recent development of super-resolution microscopy techniques have boosted fungal research.

How do the cells of filamentous fungi differ from those of other organisms?

Fungi are in many aspects identical to human cells. One important difference, however, is the presence of a rigid cell wall consisting of different carbohydrate polymers, including chitin. Because of this difference, the fungal cell wall or the biosynthesis machinery may be targets for drug development.

What biological features are conserved between filamentous fungi and animals such as humans?

Basic cell biological processes such as mitosis, meiosis, the functioning of organelles, or the principles of gene regulation, are highly conserved between humans and both filamentous

formation, however, requires the fungus to both circumvent plants' natural defences and to emit signals that actively reprogramme the plant cells to accommodate arbuscules. As molecular biology advances, and the genomes of filamentous fungi are sequenced, our understanding of the precise molecular nature of these interactions will unfold.

FROM FUNGI TO FURTHER AFIELD

The diverse and enlightening findings of this high-profile programme have implications far beyond the fungal kingdom. The methods used – particularly novel ways of imaging microscopic and rapidly-changing structures – have the potential to revolutionise studies of subcellular processes across the living world. Professor Fischer and his collaborators are finally bringing the filamentous fungi from the soil firmly into the limelight.

Detail

RESEARCH OBJECTIVES

Professor Reinhard Fischer is currently studying the filamentous fungus *Aspergillus nidulans*. His primary interest in this fungus is as a model for spore and mycotoxin formation and the effect of environmental factors on its growth. Professor Meritxell Riquelme is studying the mould *Neurospora crassa* and is interested in the cellular components enabling indefinite hyphal extension.

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BIO

Before becoming Professor for Microbiology at the University of Karlsruhe, Prof Dr Reinhard Fischer studied for his PhD in Microbiology at the University of Marburg. He has represented the DFG as a panel member since 2012 and sits on the editorial boards for a number of well-known scientific journals, including Molecular Microbiology, mSphere and mBio. Prof Dr Meritxell Riquelme completed a BS in Biology at the University of Barcelona, Spain and a PhD in Microbiology at the University of California, Riverside. She has worked at the Scientific Research Center and Higher Education of Ensenada (CICESE) in Baja California, Mexico since 2004.

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