

# The importance of fungi and mycology for addressing major global challenges\*

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**Abstract:** In the new bioeconomy, fungi play a very important role in addressing major global challenges, being instrumental for improved resource efficiency, making renewable substitutes for products from fossil resources, upgrading waste streams to valuable food and feed ingredients, counteracting life-style diseases and antibiotic resistance through strengthening the gut biota, making crop plants more robust to survive climate change conditions, and functioning as host organisms for production of new biological drugs.

This range of new uses of fungi all stand on the shoulders of the efforts of mycologists over generations: the scientific discipline mycology has built comprehensive understanding within fungal biodiversity, classification, evolution, genetics, physiology, ecology, pathogenesis, and nutrition. Applied mycology could not make progress without this platform. To unfold the full potentials of what fungi can do for both environment and man we need to strengthen the field of mycology on a global scale.

The current mission statement gives an overview of where we are, what needs to be done, what obstacles to overcome, and which potentials are within reach. It further provides a vision for how mycology can be strengthened:

The time is right to make the world aware of the immense importance of fungi and mycology for sustainable global development, where land, water and biological materials are used in a more efficient *and* more sustainable manner. This is an opportunity for profiling mycology by narrating the role played by fungi in the bioeconomy. Greater awareness and appreciation of the role of fungi can be used to build support for mycology around the world. Support will attract more talent to our field of study, empower mycologists around the world to generate more funds for necessary basic research, and strengthen the global mycology network. The use of fungi for unlocking the full potentials of the bioeconomy relies on such progress. The fungal kingdom can be an inspiration for even more.

**Key words:**

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## INTRODUCTION TO THE IMPORTANCE OF MYCOLOGY

Applied mycology has traditionally focused on areas where fungi cause damage. Highly advanced mycological research has generated knowledge, conceptual understanding and insight within fungal plant diseases, human mycoses, indoor climates, and decays in wood, feed and food. There is also extensive knowledge about the added value fungi contribute to beer, bread, wine, spirit, food preservation, and food taste. Much less attention has been given to fungi and fungal products for adding value for industry, agriculture, health, and pharmaceuticals. The last few decades, however, have witnessed very interesting developments regarding use of fungi for new processes, products and solutions of importance for the world, and most importantly for increased conceptual understanding

of the kingdom *Fungi* at large (Hibbett *et al.* 2007, James *et al.* 2006, Schoch *et al.* 2006).

It is now well documented, even in scaled up practice, that fungi can generate tangible and substantial value through improved resource efficiency, resulting in decreased pollution and greenhouse gas emissions. Such progress stands on the shoulders of the contributions made by mycologists over decades and even centuries to build up mycology as an independent field of science. Importantly, value added use of fungi was founded not only on applied studies but also on basic research aimed at understanding fungal biodiversity, growth, nutrition, physiology, genetics, metabolism, and ecology.

The shift from chemical processes to biological processing, achieved by using fungal (and bacterial) enzymes instead of chemical processes in industries, such as textiles, leather, paper and pulp, has significantly reduced negative impacts on the environment. Use of enzymes in the

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## THE ROLE OF FUNGI AND MYCOLOGY IN ADDRESSING MAJOR GLOBAL CHALLENGES

Fungi play an important role in addressing major global challenges. Use of fungal processes and products can lead to increased sustainability through more efficient use of natural resources. Applications range from upgrading bio-waste for value added products to use of renewable plant biomass as a substitute for oil-based products such as biochemicals, plastics, fertilizer, and fuel. Fungal inoculum, introduced into soil together with seed, can promote more robust plant growth through increasing plant uptake of nutrients and water, a robustness of importance for maintaining crop yields under climate change condition. Fungal enzymes can lead to production of food ingredients with prebiotic effects for a healthier human gut biota and hence greater resilience towards life-style diseases. Similarly, use of fungi can be a short cut to healthier animal feed and less use of antibiotics in, for example, meat production, one of the current prime sources of multiple drug resistant bacteria. Fungi are one of nature's most promising hotspots for finding new drug candidates and antimicrobials. Last but not least, fungi have interesting potential as the new way of manufacturing biological medicines and a wide spectrum of new value added bio-based products.

All such uses of fungi, fungal products and fungal processes reflect the efforts of mycologists over generations. Similar efforts lie behind work to cure and prevent life threatening human mycoses, to control mycotoxin contaminations, and to counteract fungal spoilage of materials and 'sick building' syndrome. As a scientific discipline mycology has built comprehensive understanding of the fungal kingdom: fungal biodiversity, physiology, genetics, ecology, pathogenesis, nutrition. Mycology includes understanding at the system, organismal and molecular level. This knowledge and insight constitute the platform that has given rise to uses of fungi in industry, agriculture, food and feed, medicine and health.

Mycology must grow fast beyond where it is today. The potential of fungi for a more sustainable world must be released to address global challenges of climate change, higher demands on natural resources, and the increased burden of life-style diseases. Genome sequencing was developed first for the human genome after which bacterial genomes were quickly sequenced. But mycology is catching up. Interestingly, up to now, industry makes use only of a minute portion of the fungal kingdom. Fungal biodiversity is a resource pool for the future. However, fungal diversity is endangered by loss of habitat, causing loss of species and loss of biodiversity in general.

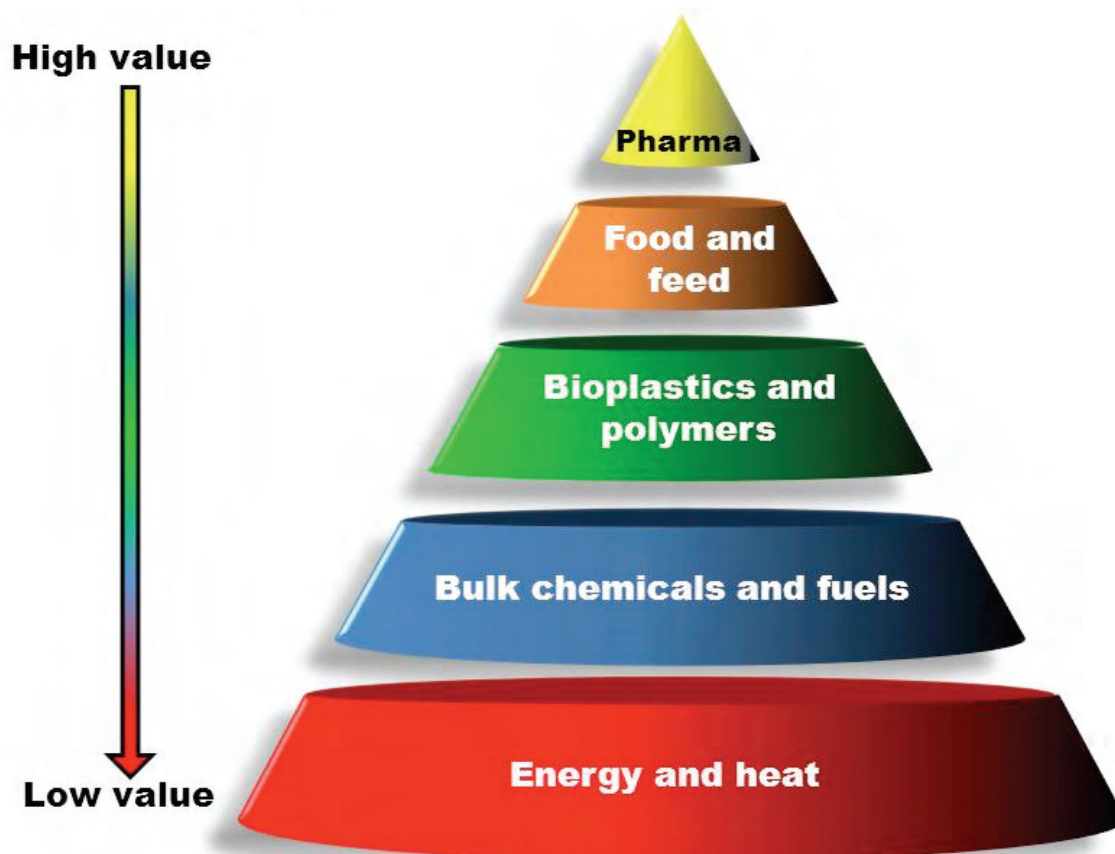
We need to stimulate mycology globally and work more efficiently together to take good care of this diversity and unlock the full potential of the fungal kingdom for future use all over the world. The discipline of mycology needs to be developed to a stage where it can recruit talent for the new generation of mycological researchers and for building the skills needed for the world to change towards the new and more sustainable bioeconomy.

In summary what is needed is: Increased *understanding of the fungal kingdom*, phylogeny and phylogenomics as a basis for understanding the fungal life-form generally, and for expanding the exploitation of fungal biodiversity for more value added uses; the *mycological platform*, building mycological know how and skills in all parts of the world. There is a global need for the bioeconomy, for increased resource efficiency and upgrading of biowaste to healthier food and feed ingredients, materials and fuel; a stronger *global mycology network*, including globally distributed databases (embracing genotypic and phenotypic data), improved opportunities for networking activities, talent recruitment, research education, and for broadening the *IMA activities* to real global inclusiveness and perspective; *Open access mycology*, a new concept where cultures and information go together to support knowledge dissemination, enabling distributed uses of fungi for upgrading bio-waste resources to higher value; a concerted effort to build a stronger focus on the *role of fungi in nature*, how they interact with substrate and other organisms, including global research efforts on the fungal secretome, as a basis for increased collaboration between academia and industry within the field of mycology; revisiting the *traditional use of microbial fungi for food*, the advanced mycological heritage, revitalized through molecular studies, giving insight in use of microbial consortia for food processing to inspire also the next generation of biobased products; and not the least: excellence in *teaching of mycology*, to be developed in a global scale to provide a better platform for recruitment of talent, for stimulating a fascination of fungal life, and for building broader skills for the bioeconomy to unfold.

food and feed industry, such as animal feed, baking, brewing, and wine and juice, has significantly improved what we get out of biological raw materials. Microbial enzymes added to detergents, washing laundry clean even at low temperatures, has significantly reduced CO<sub>2</sub> emissions. The newest chapter in the industrial biotechnology era is to substitute fossil resources with renewable resources (Fig. 1).

The focus was initially on enzymatic conversion of wheat straw and corn stover to bioenergy. The current trend is to expand the types of biomass exploited, going beyond the use of crop residues alone and including agroindustrial waste (side streams and by-products) to

produce higher value products such as bio-chemicals, biomaterials, food and feed ingredients, and circulating the micronutrients back to the soil. In other words, not *only* converting biomass to lower value bioenergy bulk products. This entire global endeavor builds almost exclusively on fungal enzymes produced on a large scale by fermentation of filamentous fungi, with recombinantly expressed fungal genes. Production hosts of choice were primarily *Aspergillus oryzae*, *A. nidulans* and *Trichoderma* species. Surprisingly the enzyme genes used are still almost all from a rather narrow selection of ascomycete and basidiomycete genera.



**Fig. 1.** The value pyramid of biomass conversion: At the bottom, with lowest value, is the bulk use of biomass for combustion, making heat and electricity. Next layer is using biomass for biofuel, a much needed renewable alternative to fossil transport fuel. Further up the biomass value pyramid, is production of specialized and higher value products (materials, chemicals, feed, food and pharma). Such products are not only substituting for fossils but also making use of the complex structures of new biomass, making products not possible to make from fossilized biomass. All uses except for burning, gasification, etc., of the biomass involve use of microbial (primarily fungal) conversion and upgrade. Courtesy of Peter Westermann.

In order to take these next steps, we must develop mycological research even more. We need to tell this story and make the world aware of the immense importance of fungi and mycology for sustainable global development, where land, water and biological materials are used in a more efficient *and* more sustainable manner. This offers us opportunity also for profiling mycology by narrating the role played by fungi and fungal enzymes in the bioeconomy. Greater awareness and appreciation of the role of fungi can be used to build support for mycology around the world. Support will attract more talent to our field of study, empower mycologists around the world to generate more funds for necessary basic research, and strengthen the global mycology network.

### THE IMPORTANCE OF MYCOLOGICAL RESEARCH FOR FURTHER BIOLOGICAL SOLUTIONS TO IMPORTANT PROBLEMS

Discovery of novel fungal products for specific industrial needs, builds on basic studies of fungal biodiversity, and on using the experimental molecular mycology tool box

for identifying potentially interesting genes and proteins, synthesis pathways, and metabolites. For industrial use it is the secretome of the fungi which is targeted for both metabolite and enzyme discovery<sup>1</sup>.

Most molecular studies within fungi have focused on what the fungi are and only less on what fungi do and how they interact with other organisms and substrates. The molecular era first focused on elucidating phylogenetic relationships and species identification. Now the time is ripe for intensifying the study of the secretome with regard to composition, development, and evolution. Molecular studies of the secretome (genomics and transcriptomics of secreted proteins) will add new conceptual understanding of the role of the secretome in substrate specialization and organismal speciation. We will learn more about the mechanisms of secretome evolution, going beyond mutation and selection. This evolution was most likely influenced by mechanisms such as series of gene copy/gene loss and retention of the protein with best fit for substrate utilization and growth; or

<sup>1</sup>Intracellular enzymes are too costly to recover from large scale fermentations for industrial purposes.

by horizontal transfer, the latter being expected to be found especially in closely interacting ecological niches such as the animal rumen and the phyllosphere.

Discovery of enzymes suitable for industrial processes draws upon mycological research within a wide range of mycological disciplines. These include biodiversity, fungal physiology and ecology, molecular biology, protein chemistry, enzymology and assay technology, and not least experimental mycology, involving yeasts (as screening hosts) and filamentous fungi (as gene donors and protein production organisms). Progress in all such fields has made it possible within a rather few years to find the enzyme cocktail needed to break down recalcitrant lignocellulose to a monomer sugar platform (Carvalho *et al.* 2008, Kubicek *et al.* 2014, Martinez *et al.* 2009).

Until recently, the short cut from enzyme discovery to testing most promising enzyme proteins employed the following steps: optimizing the enzyme discovery by identifying and choosing the conditions (substrate, temperature, aeration) most conducive to enzyme expression, harvesting the induced fungal biomass, extracting total RNA, recovering the mRNA fraction, converting to cDNA, constructing cDNA libraries, and screening the libraries on specific assay plates, allowing for identification of positive transformant yeast colonies, recombinantly expressing a full length gene, resulting in a secreted enzyme, with the activity indicated by the assay plate used (Dalbøge & Lange 1998, Fowler & Berka 1991).

In the last few years, choice of technology for enzyme discovery has moved towards a strategy focusing on genome or transcriptome sequencing instead of (cDNA) library construction and screening (Huang *et al.* 2014). This new sequencing approach allows for higher speed discovery and lower cost experimental work. However, it also leads to the need for additional effort within protein expression. For a particular genome sequence there is much less information about whether it can easily be expressed recombinantly and whether the result of the expression will be a functional enzyme. With the cDNA screening strategy, you have a yeast clone where you know you have a full length gene, that the gene is expressed; and that expression leads to a secreted, functional gene, active in a specific enzyme assay.

Similarly, scaled up production of secreted fungal enzymes rests on experimental results, conceptual understanding and insight achieved through basic mycological research within the fields of genetics, molecular biology and protein expression – embracing also optional expression of basidiomycete, zygomycete and chytridiomycete proteins in ascomycete hosts and production organisms. For improved biological production we need a strengthened basis in fungal phylogeny, progeny trees, protein expression, fermentation technology, and not least more knowledge about the biosynthetic pathways of mycotoxins and mycotoxin occurrence and toxicity (Frisvad & Samson 2004). Besides such mycological research, large scale production is also based on knowledge within the field of chemistry and on fungal growth, including modelling studies for optimization of growth conditions.

What is often forgotten when trying to see the big picture is that the success of industrial biotechnology using fungi and fungal products builds primarily on the unique

and highly efficient function of the tip cells of filamentous fungi, efficiently secreting proteins produced by the rapidly growing mycelial biomass. Advanced bio-imaging studies have provided valuable insight into this exciting field of biology; more bio-imaging studies across fungal phyla could give insight in the variation found within the fungal kingdom (Harris *et al.* 2005).

## INSPIRATION AND LEARNING FROM NATURE

The need for more types of enzyme blends for efficient decomposition of further kinds of lignocellulosic biomass (not only wheat straw and corn stover) has revitalized the entire field of studies on fungal degradation of lignocellulose, including ascomycetous saprophytes and basidiomycetous white rot and brown rot fungi. This quest has also led to new studies of ecological habitats, where biomass conversion takes place in nature, developed over evolutionary time. The enzymes from the termite larvae gut channel and from the cow rumen, investigated by activity screening of cDNA meta-libraries, were already studied decades ago by some biotechnology companies. Description of the biomass conversion of the ectomycorrhizal *Paxillus involutus* (Rineau *et al.* 2012), studied by mass spectrometry (MS) for the documentation of bioconversion and Transposon Assisted Signal Trapping (TAST) of the cDNA library for discovery (Hamann & Lange 2006, Rineau *et al.* 2012), gave new insights into the possible dual function of this ectomycorrhizal fungus.

A most interesting meta-study examined the enzymes produced and secreted in the fungus garden of the leaf cutter ant (Grell *et al.* 2013). This showed that *Leucoagaricus gongylophorus*, farmed by leaf cutter ants, expressed the entire spectrum of enzymes needed for breaking down the cellulose fibres of the green leafy biomass which the ants bring to the fungal garden in their nest (Fig. 2). The ants pretreat by chewing the biomass. The fungus expresses the needed regime of enzyme proteins. The enzymes even survive (in an intact and active form) passage through the gut channel of the ants. Redistribution of the enzymes to the newly harvested leafy biomass is achieved by the ants placing their enzyme-holding fecalia on newly harvested leaves on the top of the fungus garden. This entire sophisticated mutualistic system has been comprehensively described (Kooij 2013).

In a recent review of the prominent role of fungal enzymes for the success of the leaf cutter ants (Lange & Grell 2014), the system is described from a new perspective that sees the fungal partner in the symbiosis not merely as being passively farmed but also as an active partner. In this interpretation the *Leucoagaricus* fungus is an example of a fungal adaptation path towards developing attractants to recruit insects to disperse the fungal spores (as, for example, flies are attracted to the spore gleba of *Phallus impudicus* and thus lured into disseminating the fungal spores). The ant-farmed *L. gongylophorus* benefits from the association by having an assured food supply, by having its colony weeded for intruders, and by being protected from parasite populations (Seifert *et al.* 1995). Lange & Grell (2014) reviewed the literature and concluded that the mutualism functions on



**Fig. 2.** Learning from Nature's "green biorefinery": Leaf cutter ants carry pieces of green leaves to the fungus garden (**upper**), feeding the fungal symbiont, *Leucoagaricus gongylophorus*, farmed in the fungal garden in the subterranean ant nest (**lower**; laboratory culture of Jacobus Boomsma). The fungus produces swollen tipped cells, filled with proteins and sugars, the gongylidia, organized in staphylae. The gongylidia are picked by the ants for feeding the ant colony with protein and sugar rich feed. Bottom line is that this successful and complex society, where fungal enzymes convert green leaves into accessible, highly nutritious fungal biomass provide basis for one of the most successful life forms on earth. Photo: Henrik H. De Fine Licht.

the molecular level due to the fungal enzymes and on the organismal level due to the fungal gongylidia (swollen hyphae filled with proteins and sugars), serving as highly nutritious food for the ants. The ants collect, distribute and chew (pretreat) the leafy biomass, and remove "garbage". It is the continuous supply of highly nutritious fungal gongylidia organized in bunches, the staphylae, readily harvestable by ants, which provides the basis for the ant colonies to grow to

such size and societal complexity. The global success of the ants is based on the fungal enzymes, the nutritional value of the fungal mycelium and the efficiency of the filamentous fungus growth and tip cells.

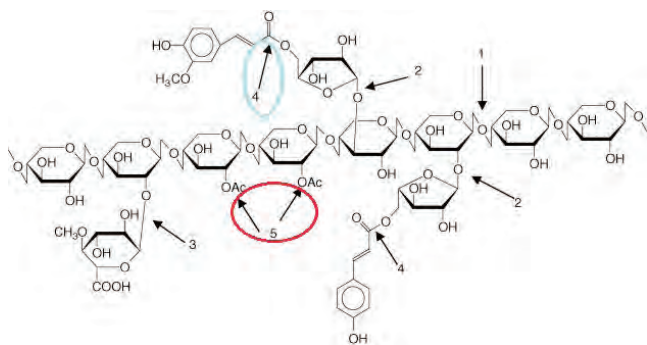
We can learn from nature about how to construct biorefinery processes. The leaf cutter ant colony including the fungal garden can be seen as the archetype of a green biorefinery. The cow rumen can be seen as a biorefinery for decomposing lignocellulosic straw, the yellow biorefinery. And the termite larvae gut channel can be seen as a biorefinery decomposing lignocellulosic woody materials. Interestingly, we also see that fungi are responsible for most of the lignocellulytic enzymes in most such biomass decomposing habitats in nature. However, bacteria are always found in such biomass conversion niches and most likely are also playing a role in overall biomass conversion.

### THE NEW POTENTIAL OF FUNGI AND FUNGAL PRODUCTS FROM BIOREFINERIES

There are many new examples in the pipeline of both academic and industrial research for upgrading the value of biomass and waste. One of the most interesting is directly inspired by the leaf cutter ant/fungal garden mutualism. In the fungal garden of the ants, the fungus itself serves as highly nutritious animal feed! Feeding the projected nine billion people on Earth by 2050 (United Nations' World Population Prospects report) will put enormous pressure globally on available arable land, water, and nutrition. Right now at the global scale we use around 72 % of all arable land for the production of animal feed. If more animal feed is produced based on the huge amounts of bio-waste we lose and discard along the chain from crop to food, and from the field to end user, we could release more land for biodiversity conservation and for food production (FAO 2013).

The latest research paves the way for making even more efficient use of the potential of biomass. First, cellulose fibres are broken down to sugar monomers by fungal enzymes. Next in the value upgrade is using the sugar platform for growing microbes which produce building blocks for chemicals and for biopolymers, such as bioplastics. The lignin will be developed into binders and materials (still to be developed). And the hemicellulose polymer is processed by fungal enzymes for recovery of C<sub>5</sub> sugar oligosaccharides with prebiotic activity for a more healthy gut microbiota. This means that now we do not just break down nature's complexity for building up new complexity from the sugar platform. We also recover nature's complexity, for example proteins and hemicelluloses, to make new and upgraded products, food, feed, and materials with new properties and value adding functionalities.

Fig. 3 shows some of the specific and highly specialized functions of fungal enzymes, currently under evaluation for industrial processing of hemicellulose into high value prebiotic food and feed ingredients. The ambition is to develop food ingredients which can make people more robust against life-style diseases and to develop animal feed for non-ruminant animals such as pigs with the prebiotic effect to improve metabolism. This gives better welfare and less need for antibiotic treatment – and limiting antibiotic



**Fig. 3.** The hemicellulose plant cell wall polymer, arabinoxylan, is degraded by many different and highly specialized fungal enzymes in nature: 1. endoxylanases; 2.  $\alpha$ -L-arabinofuranosidases; 3. glucuronidases; 4. ferulic acid esterases; and 5. acetyl xylan esterases. Ongoing research aims to use such specific fungal enzymes to modify the arabinoxylan, a sub stream from lignocellulose biorefinery, into  $C_5$  sugar oligosaccharides with a prebiotic effect, stimulating the healthy gut fungal and microbial populations of humans and other animals. Modified from Chavez *et al.* (2006).

use lowers the risk of runaway resistance to antibiotics. All this is achievable by converting waste materials using fungal enzymes.

The biotech industry has a good track record of using not just plant biomass but also fungal biomass directly as feed. The yeast biomass or “vinasse” left over from large scale production of insulin was used as animal feed for pig production in Denmark for many decades. The pigs loved it! Now a new approach is proposed. Transform sanitized household bio-waste into a fermentation medium, and use it as substrate for fungal growth. From such a system the fungus biomass in itself, as a yeast cream, can be used as animal feed, which through fungal and bacterial biotransformation is one step removed from waste. With the available nitrogen supply the fungus will be able to develop into new nutritious protein-rich animal feed. Focused efforts will have to be made to optimize such a system, including optimizing the waste to fermentation medium process, for example by mixing different complementary and matching waste streams. Research efforts have also been initiated with the objective of strain improvement to achieve an even higher nutritional value of the resulting fungal biomass. Two groups of fungi are being studied for this purpose: yeasts (*Saccharomyces cerevisiae* or *Candida utilis* nonallergenic mutants) and basidiomycetes such as those grown by termites (*Termitomyces*, Fig. 4) (Aanen *et al.* 2009; Nobre *et al.* 2010), ants (*Leucoagaricus*), or humans (*Pleurotus*). Additional molecular studies can lead to even higher levels of fungal protein content and bio-accessibility. The strain improvement of such “Waste2Value Fungi” can also be expanded to include strains which concentrate micronutrients, such as phosphorus, iron and selenium, further enhancing the nutritional value of the fungal animal feed.

Another potential way to extend conversion of biowaste for producing food and feed ingredients is to include animal derived materials, such as fish (by-catch and waste) as well as slaughter house waste such as pig bristles and chicken feathers (Fig. 5). New discovery efforts are underway to

find and develop fungal (and bacterial) enzymes, primarily proteases, for converting such hitherto unexploited protein rich waste streams, for example obtaining otherwise inaccessible protein from the keratin of pig bristles and chicken feathers decomposed by enzymes from *Onygena corvina* (Huang, Busk & Lange, unpubl.) to produce nutritious food and feed ingredients, without using more land or making further inroads into naturally occurring or farmed fish populations. Another area where we need new enzymes is for the conversion of leftover press cake from production of plant oil from olive, palm tree, sunflower, rape, etc., into bio-accessible protein-rich animal feed. This represents a substantial underexploited source of protein for both animal and human consumption.

## NEW META-LEVEL DISCOVERIES

The affordable price of genome sequencing has made meta-studies of entire ecological niches doable, for example by metatranscriptomic or metagenomic sequencing, where an entire niche is handled as if it were one organism. This approach enables inclusion of non-culturable organisms as well as all kinds of auxiliary enzymes we still are ignorant of. Another approach under development is to start including more than just ascomycetes and basidiomycetes in the enzyme discovery efforts. Interesting results are being



Species	Protein (% DW)
Button mushroom ( <i>Agaricus bisporus</i> )	35.7
Oyster hat ( <i>Pleurotus ostreatus</i> )	27.7
Chanterelle ( <i>Cantharellus cibarius</i> )	22.3
'Chicken mushroom' ( <i>Termitomyces spp.</i> )	30.1-48.0

**Fig. 4.** Learning from nature: the specialized basidiomycete *Termitomyces titanicus* (Agaricales) grows in subterranean termite nests. It can grow to form massive and impressive basidiomes, used as a human delicacy (above). The benefit to the termites – even without having developed the sophisticated farming procedure – is accessibility to protein rich feed. The percentages of protein in edible basidiomycetous fungi are high (measured as % of total dry weight; (below); an extraordinarily high protein content has been recorded for *Termitomyces* species. In future we will be able to make biorefineries by growing fungi on household waste and use the protein rich fungal biomass for animal feed. Photo and table: Duur Aanen.



**Fig. 5.** *Onygena* species. Non-pathogenic species of *Onygenales*, are specialized in breaking down the keratin found in feather, hooves, and horn. The keratin is composed of proteins, bound in a non-bio-accessible form. Among the large number of different proteases produced by *O. corvina*, we discovered that just three enzymes, belonging to two types of protease families, are sufficient to breakdown both feather and pig bristles. The picture shows *O. equina* growing on horn, but not on the skull (Northern Ireland, 2012). Photo: Jens H. Petersen.

gained right now from chytrids and zygomycetes, notably *Entomophthorales* (Grell *et al.* 2011) and *Mucorales* (Huang *et al.* 2014).

### NEW SEQUENCE ANALYSIS TOOLS, MAKING ORDER AND SENSE OUT OF CHAOS

As outlined above, the need for new enzymes is huge. Building the bioeconomy will include significant efforts in enzyme discovery to facilitate the diversification of substrates to be upgraded and of products to be developed from the biorefinery. Such discovery efforts will simultaneously result in massive accumulation of underutilized genome sequencing data (Murphy *et al.* 2011, Grigoriev *et al.* 2011). The vision is straightforward: if we become better at predicting functions from sequences, we could significantly shorten and sharpen the enzyme discovery process. We could go directly from sequence to the subgroup of enzyme genes we would like to screen to identify the one with the highest potential for that specific biomass conversion process. With that specific goal in mind, Busk & Lange (2013) developed a new sequence analysis approach, Peptide Pattern Recognition (PPR), giving a function-based protein classification by recognition of short, conserved peptide motifs.

PPR, the first non-alignment based sequence analysis methodology, predicts function directly from sequence with an accuracy of 80–97 % (Busk & Lange 2013). The method was developed further to include a regime by which a large sequence database generated through PPR can be mined for the presence of, for example, glycohydrolases, resulting in a list of genes referred to specific CAZy protein families (<http://www.cazy.org/>; Cantarel *et al.* 2009). This list can be transformed into one providing an overview of all the functions (illustrated as a list of EC numbers) found in the secretome of that ecological niche. For each function, the protein families that have those functions, often more than one type of protein family for each EC function, can be specified.

### FUNGAL INOCULUM FOR STRONGER AND MORE ROBUST PLANT GROWTH

Another highly promising field for new microbial and fungal products is the use of inocula for strengthening crop plants, making the plants more robust to abiotic stress and more efficient with regard to water and nutrient utilization. Since the days of the green revolution, plant breeding has taken place almost in isolation (except for breeding for increased tolerance against certain plant diseases and pests). Now, advances in the field of fungal and microbial products for agriculture is making it possible for plant breeders, mycologists, and microbiologists, to work together to find the combination of plants and other organisms which will provide farmers and the world with more robust and resilient agriculture. At a time when climate change challenges agriculture in many parts of the world, and where water and nutrition are at a minimum in many places, the combinations of fungi, microbes, and plants can provide opportunities for significant progress in global food, feed, and biomass production.

It will be interesting to follow developments in this area. Again, a new field is emerging that builds on a platform of knowledge generated through the combined efforts of public and private mycological research, where fungi are seen as having the potential to contribute significantly towards a more sustainable world. This is therefore a highly interesting area for contributions from mycologists specialized in endophytes, mycorrhizas, *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium*, soil fungi, and consortia where bacteria and fungi work together to produce efficient and optimized systems.

One product is already available on the market which increases phosphate availability, “JumpStart” (Novozymes 2012; <http://www.bioag.novozymes.com/>), which is a phosphate inoculant containing the naturally occurring non-GMO soil fungus *Penicillium bilaii*. The fungus colonizes and grows along plant roots, releasing organic compounds that, in turn, release the “bound” mineral forms of otherwise less available soil and fertilizer phosphate, which thus becomes immediately available for the crop plant.

## NEW BREAKTHROUGHS NEEDED

Next in demand are regimes of enzymes active under low temperature conditions and enzymes active and stable at high temperatures for decomposition of both plant and animal derived biomasses. Here, mycologists specialized in extremophiles can contribute significantly (Zajc *et al.* 2013, Pitt & Hocking 2009, Gleason *et al.* 2010). Expansion of commercialized enzymes from highly extreme ecological niches, such as extremophilic archaea, with enzymes active and stable above 100°C, also calls for new breakthroughs in recombinant expression. Could intensified studies of the archaean splicing mechanisms and protein expression lead to a breakthrough in the development of fungal production hosts for heat stable secreted enzymes from these microorganisms? Improved expression of basidiomycete genes in ascomycetous expression hosts, such as yeasts, also builds on an improved understanding of the variations in protein expression mechanisms within the fungal kingdom. Enzymes to convert the proteins locked into keratin in feathers and bristles into bioavailable protein resources would pave the way for using such animal waste products for highly nutritious animal feed (Huang, Busk & Lange, unpubl.).

There are discoveries to be made of new kinds of antibiotics from fungal hotspots for antibiotics. For example insect pathogenic fungi, which have developed ways to reserve the entire insect carcass for themselves, or cellulose/sugar fungi that protect the dimer and monomer sugars against piggy-backing bacteria, preventing them from 'stealing' the sugars after the fungi have produced all the enzymes to break down the lignocellulose. Discovery of novel drug candidates with new modes of action and central nervous system active metabolites from fungi, which manipulate insects to adopt behavioral patterns that optimize the chances for dispersal of fungus spores formed on the insect after the insect dies (e.g. ants and flies programmed to climb to the top of grass and cling there until death; or the house-fly which is manipulated to settle on a window glass pane and spread out its wings to maximize the area for fungal spore production and release). Maybe such research efforts can reveal new concepts for neurosignalling, induced in animals, based on stimuli given by fungal metabolites or proteins, but which may also be of relevance for increased understanding of the human central nervous system?

New higher value products can also be developed from fungal "vinasse" biomass left over from large scale biotech production. This is important because there will be a steep increase in amounts of fungal biomass available for upgrade as the technology of biomass conversion in biorefineries develops. In nature, complex patterns and mechanisms of collaboration are expected to occur especially between fungi and bacteria. This is a most interesting area for basic studies, and has the potential to reveal organized consortia we have not even dreamt about so far. A model to use as a starting point for further studies is the enrichment consortium of waste water treatment (Nielsen *et al.* 2009, Albertsen *et al.* 2011). In the future, when complex organic substrates are to be broken down to complex products to solve challenging problems, fungal and microbial consortia could provide a short-cut to a solution.

Last, but most importantly, further studies are urgently needed to prevent, control and cure serious human mycoses caused by *Coccidioides* species (Taylor 2006). The number of fatalities caused by this fungal disease, especially in Africa, approaches that caused by tuberculosis and malaria, but far less effort has been invested in preventing and curing human mycoses. Perhaps basic studies of the secretome (including substances bound to the outer wall structures), and new molecular insights, instruments and technologies could bring us a step further in this very difficult significantly under prioritized area of health and pharmaceutical investment.

## HOW TO STRENGTHEN INTERNATIONAL MYCOLOGY FOR A BIOBASED FUTURE

It is evident from the above descriptions that investment in strengthening mycology globally is worthwhile. The following efforts are urgently needed to enable this to occur:

- (1) Understanding the fungal kingdom, through phylogeny and phylogenomics: support for global participation and global coverage of strains used for resolving the Fungal Tree of Life.
- (2) Strengthening the mycological platform for classification, identification and strain collection: support implementation of one name one fungus nomenclature; recollection and sequencing of fungi not included in molecular database, including neo- or epitypification where appropriate; develop principles and systems ready for capturing and categorizing sequence discoveries of novel fungal genes not originating from any known species.
- (3) Building a global mycology network: develop globally distributed databases, with global input and access; establish a visiting professor programme and a young investigator international exposure programme; establish an IMA delegate participation programme, allowing all regions to participate equally in IMA ExCo meetings and other organizational IMA activities.
- (4) Open access mycology: establish global access/regional collections of bioeconomically-relevant strains and biological materials, unique in that strains and materials are accompanied by searchable information about uses, recipes/protocols on safe and fast use for advancement of the bioeconomy, e.g. for upgrading biowaste to new biobased products.
- (5) The role of fungi in nature, interactions, and the secretome: provide global assistance to mining sequence databases, faster and more function targeted, for recognizing functional proteins and metabolites; strengthen global discovery and stimulate collaboration between industry and academia in all parts of the world.
- (6) Mycological heritage programme: we need to know and tell the fantastic history of how fungi have been used since ancient times, the mycological human heritage revitalized through molecular studies and tests of efficacy of traditional uses of fungi, also the use of fungal and microbial consortia for food processing to inspire or provide leads for the next generation of biobased products?



(7) Excellence in teaching and communication: to share best practice, striving for excellence to stimulate and inspire the next generation of mycologists and to attract talent for further development of mycology, including the production of web-based training aids.

## REFERENCES

- Aanen DK, de Fine Licht HH, Debets AJM, Kerstes NAG, Hoekstra RF, Boomsma JJ (2009) High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites. *Science* **326**: 1103–1106.
- Albertsen M, Hansen LBS, Saunders AM, Nielsen PH, Nielsen KL (2011) A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal. *ISME Journal* **6**: 1094–1106.
- Busk PK, Lange L (2013) Function-based classification of carbohydrate-active enzymes by recognition of short, conserved peptide motifs. *Applied and Environmental Microbiology* **79**: 3380–3391.
- Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Research* **37**: D233–D238.
- Carvalho F, Duarte LC, Gírio FM (2008) Hemicellulose biorefineries: a review on biomass pretreatments. *Journal of Scientific & Industrial Research* **67**: 849–864.
- Chávez R, Bull P, Eyzaguirre J (2006) The xylanolytic enzyme system from the genus *Penicillium*. *Journal of Biotechnology* **123**: 413–433.
- Dalbøge H, Lange L (1998) Using molecular techniques to identify new microbial biocatalysts. *Trends in Biotechnology* **16**: 265–271.
- FAO (2013) *Food Wastage Footprint: impacts on natural resources. Summary Report*. Rome: Food and Agriculture Organization (<http://www.fao.org/docrep/018/i3347e/i3347e.pdf>).
- Fowler T, Berka RM (1991) Gene expression systems for filamentous fungi. *Current Opinion in Biotechnology* **2**: 691–697.
- Frisvad JC, Samson RA (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of the food and air-borne terverticillate penicillia and their mycotoxins. *Studies in Mycology* **49**: 1–173.
- Gleason FH, Schmidt SK, Marano AV (2010) Can zoospore true fungi grow or survive in extreme or stressful environments? *Extremophiles* **14**: 417–425.
- Grigoriev IV, Cullen D, Goodwin SB, Hibbett D, Jeffries TW, et al. (2011) Fueling the future with fungal genomics. *Mycology* **2**: 192–209.
- Grell MN, Jensen AB, Olsen PB, Eilenberg J, Lange L (2011) Secretome of fungus-infected aphids documents high pathogen activity and weak host response. *Fungal Genetics & Biology* **48**: 343–352.
- Grell MN, Linde T, Nygaard S, Nielsen KL, Boomsma JJ, Lange L (2013) The fungal symbiont of *Acromyrmex* leaf-cutting ants expresses the full spectrum of enzymes to degrade cellulose and other plant cell wall polysaccharides. *BMC Genomics* **14**: 928.
- Hamann T, Lange L (2006) Discovery, cloning and heterologous expression of secreted potato proteins reveal erroneous pre-mRNA splicing in *Aspergillus*. *Journal of Biotechnology* **126**: 265–276.
- Harris SD, Read ND, Roberson RW, Shaw B, Seiler S, Plamann M, Momany M (2005) Polarisome meets Spitzenkörper: microscopy, genetics, and genomics converge. *Eukaryotic Cell* **4**: 225–229.
- Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, et al. (2007) A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**: 509–547.
- Huang Y, Busk PK, Grell MN, Zhao H, Lange L (2014) Identification of a  $\beta$ -glucosidase from the *Mucor circinelloides* genome by Peptide Pattern Recognition. *Enzyme and Microbial Technology* **67**: 47–52.
- James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, et al. (2006) Reconstructing the early evolution of *Fungi* using a six-gene phylogeny. *Nature* **443**: 818–822.
- Kooij PW (2013) *Fungal adaptations to mutualistic life with ants*. PhD thesis, Copenhagen University. (<http://www.pwkooij.com/PWKooij/Publications.html>).
- Kubicek CP, Starr TL, Glass NL (2014) Plant cell wall-degrading enzymes and their secretion in plant-pathogenic fungi. *Annual Review Phytopathology* **52**: 427–451.
- Lange L, Grell MN (2014) The prominent role of fungi and fungal enzymes in the ant-fungus biomass conversion symbiosis. *Applied Microbiology and Biotechnology* **98**: 4839–4851.
- Martinez D, Challacombe J, Morgenstern I, Hibbett D, Schmoll M, et al. (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *Proceedings of the National Academy of Sciences, USA* **106**: 1954–1959.
- Murphy C, Powlowski J, Wu M, Butler G, Tsang A (2011) Curation of characterized glycoside hydrolases of fungal origin. *Database*: bar020 doi:10.1093/database/bar020.
- Nielsen PH, Kragelund C, Seviour R J, Nielsen JL (2009) Identity and ecophysiology of filamentous bacteria in activated sludge. *FEMS Microbiology Reviews* **33**: 969–998.
- Nobre T, Eggleton P, Aanen DK (2010) Vertical transmission as the key to the colonization of Madagascar by fungus-growing termites? *Proceedings of the Royal Society of London, Biological Sciences* **277**: 359–365.
- Pitt JI, Hocking AD (2009) *Fungi and Food Spoilage*. 3<sup>rd</sup> edn. New York: Springer.
- Rineau F, Roth D, Shah F, Smits M, Johansson T, et al. (2012) The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. *Environmental Microbiology* **14**: 1477–1487.
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006) A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* **98**: 1041–1052.
- Seifert KA, Samson RA, Chapela IH (1995) *Escovopsis aspergilloides*, a rediscovered hyphomycete from leaf-cutting ant nests. *Mycologia* **87**: 407–413.
- Taylor JW (2006) Evolution of human-pathogenic fungi: phylogenies and species. In: *Molecular Principles of Fungal Pathogenesis* (Heitman J, Filler SG, Edwards JE jr, Mitchell AP, eds): 113–132. Washington DC: American Society of Microbiology Press.
- Zajc J, Liu Y, Dai W, Yang Z, Hu J, Gostinčar C, Gunde-Cimerman N (2013) Genome and transcriptome sequencing of the halophilic fungus *Wallemia ichthyophaga*: haloadaptations present and absent. *BMC Genomics* **14**: 1–21.