Annual Report **2010**

Center for Microbial Biotechnology
Department of Systems Biology

Contents

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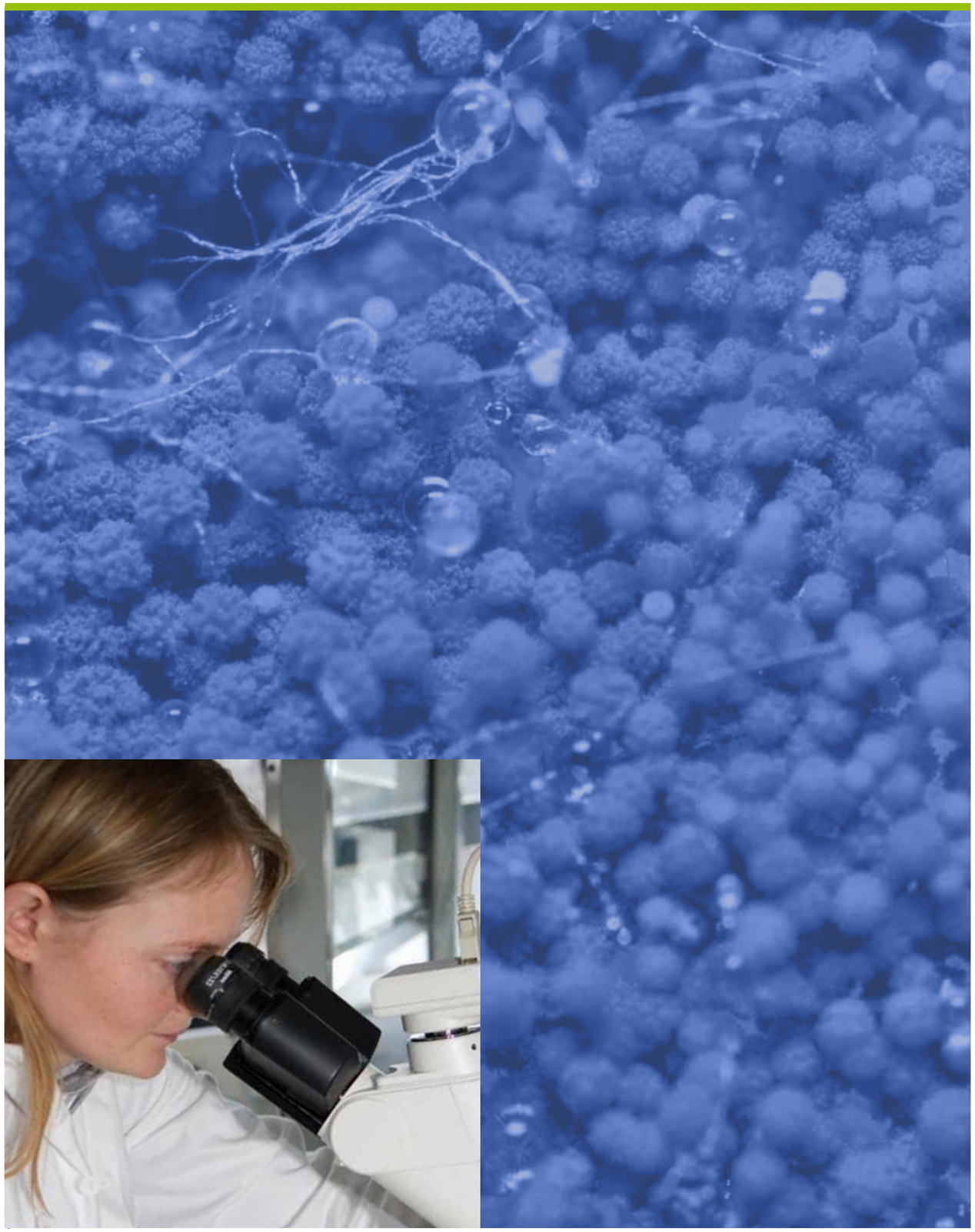


Executive Summary

Year 2010 was an important year for CMB. First and foremost, it was a year of enthusiasm around results and infrastructures. We obtained strong results from the work invested in our new emphasis on functional genomics of fungal secondary metabolite production made possible by the 2009-2013 IVC (IngeniørVidenskabeligt Center) grant from the Danish Council for Independent Research. There was also a massive setup of equipment and functionality in our two infrastructure platforms. Timing of these events is perfect for the progress of the cross-disciplinary projects laid out in the IVC and the other research activities at CMB. The following pages will describe some of these projects; here I summarise some of their bases.

The Fermentation Platform was made possible by a 30 mio. DKK grant from DTU to the Department of Systems Biology and is directed by Jette Thykær, Associate Professor at CMB. The large majority of the equipment is hosted by CMB, and CMB is so far the major user of the platform. It is foreseen that other centres at DTU will increasingly benefit from the platform already in the near future. The platform comprises 60 high-performance fermentors with volumes from 0.2 to 5 litres and much other equipment. Among the advanced analytical equipment is an off-gas analyser that by frequent shifts concomitantly can monitor all 60 fermentors. A visually very fascinating piece of equipment is a laboratory robot set up to fill, inoculate and follow many hundred parallel fermentations in microtiter plates. The robot also transports the plates into and out from the shaking incubator and the optical plate reader. The robot can also be used for other high-throughput operations, including genetic modifications in very large numbers.

The Metabolome Platform at CMB is headed by Associate Professor Kristian Fog Nielsen. In 2009 it became part of a national Metabolomics Infrastructure through a grant from the Danish governmental Globalisation fund. Operations with equipment acquired within this grant include mass spectrometry with an accuracy better than the mass of an electron, coupled to Ultra-High Performance Liquid Chromatography. This allows a dramatic rise in speed and reliability of compound identification. Among the challenges in the field of metabolomics, seen both worldwide and at CMB, is an increasing interest in quantification of intermediates in metabolic pathways, often in primary metabolism, since this will help refine the models that allow optimisation of cell factories. Metabolic half-lives of intermediates are sometimes quite short, posing challenges to quenching techniques. Recently initiated PhD projects bridging fermentation and metabolomics will deal with these developments.

The most important output from a university is candidates at all levels. While the platforms at CMB have set the stage for high-quality multidisciplinary research, they also allow for a high-quality educational environment. This is reflected by a high activity of students in our buildings who benefit from working on projects that simultaneously yield insight into several disciplines at CMB. We are proud that the departmental Teacher of the Year was from CMB (see Highlights).

The position as Director of CMB (my successor) was announced open, and an evaluation committee is at the time of writing in the process of determining who of the applicants are qualified. When my successor takes the position as Director, I will stay part time at CMB as member of the Faculty. Since 1 January 2011, the Department and I have strengthened the management of CMB by delegating personnel responsibility and other management tasks to two Vice-directors; Associate Professors Thomas O. Larsen and Uffe H. Mortensen.

While equipment and organisation are admittedly essential, it is really people who makes the difference. Year 2010 has shown us that, even after an unhappy cut in budget and staff, it is possible to maintain and even improve the enthusiasm around the projects, the cross-disciplinary collaboration, and the joy of getting the results. Even though fundraising, strategies, etc. are needed, the real source for our results and for the satisfaction associated with the usually hard work in research and education is to be found in all CMB employees and students; big thanks to everybody!

Morten Kielland-Brandt

Morten Kielland-Brandt

Director of CMB

Attention!... Calling all competencies

By Chris Tachibana for CMB

Chemists and biologists join forces to understand a versatile natural product

Holm, Klejnstrup, Nielsen, Nielsen and Rank. A law firm? A 1970s folk-rock band? No, these researchers are the core of the CMB polyketide group, a multidisciplinary consortium of chemists, microbiologists, and geneticists. Their project? To completely understand the synthesis of polyketides, which are the building blocks for the antimicrobial drugs amphotericin and erythromycin, the anticholesterol medication lovastatin, the fungal aflatoxins, and more. "It requires all the competencies we have at the centre," says Jakob Blæsbjerg Nielsen, a postdoctoral scientist on the project who is now a CMB Assistant Professor.

Geneticists figure things out by knocking out genes and seeing how the cells respond, so the CMB polyketide team started with geneticists Blæsbjerg Nielsen and Associate Professor Michael Lynge Nielsen, with Associate Professor Uffe Mortensen's group, deleting all potential genes for polyketide synthase enzymes from the filamentous fungus *Aspergillus nidulans*. "We had an organism with 32 possible genes and we wanted to know if they encoded polyketide synthases and if so, what they produced," says Lynge Nielsen, who was the group's leader on the microbiology side. "But linking genes to products is challenging." This is where the chemists, who analyze cells by making extracts and characterizing the component compounds, came in.

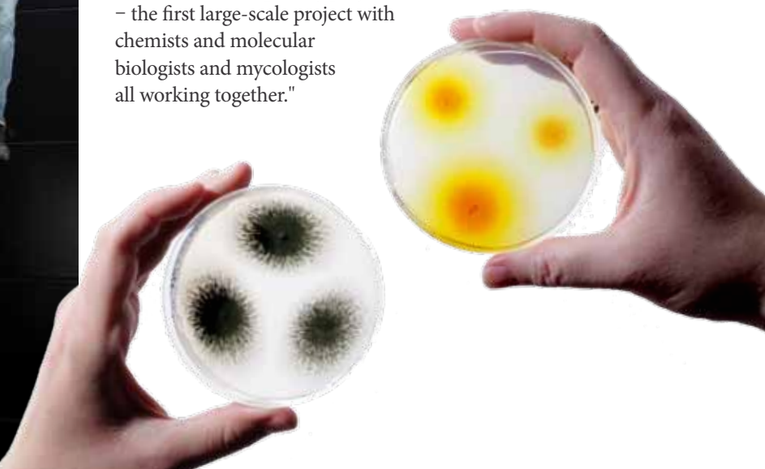
A. nidulans strains with gene deletions were grown on eight different media and handed off to chemists Christian Rank, a postdoctoral researcher with Associate Professor Thomas Ostenfeld Larsen, and Marie Louise Klejnstrup, a PhD student with Larsen and Mortensen. They did compound analysis and structure elucidation using high-pressure liquid chromatography (HPLC) and mass spectrometry to see how the chemical profiles for polyketide-like molecules changed. The profiles of the deletion strains were altered, but not in the simple way they team expected. "Ideally, we'd look at a mutant strain profile and see that one peak was gone," says Rank, "but we didn't have that scenario – ever. Usually, we saw that five other peaks came up out of nowhere." Blæsbjerg Nielsen continues, "We think this is because of crosstalk with other pathways, because when you change something, you affect the whole organism."

The polyketide group itself functioned as an organism, with a division of labour, a central command, and crosstalk between group members. This kind of interdisciplinary team is vital to tackling large-scale projects with data from genomics, proteomics, and metabolomics experiments. However, anyone who has tried to talk shop with someone from another field knows how hard detailed communication across disciplines can be. "The main challenge is to understand each other, to speak the same language," says Lynge Nielsen.

A central headquarters was crucial, says Rank. "A critical thing was putting Michael and me in the same room. It helped us understand how things are produced and how they are analysed on the other side, and that flowed out to the others." To organize a complex, multidisciplinary project, Blæsbjerg Nielsen says, "It's good to have a master plan and workflow, and to keep people involved in each step. We had a big Excel sheet with each step in the process to say when a strain was verified and was ready for chemical analysis. It was a pipeline of sorts." Clear advance expectations also help. Going into her PhD work, chemist Klejnstrup knew that, "part of my project was to work with molecular biologists." Keeping the pipeline moving was a challenge, says Lynge Nielsen, since all the team members were also working on other projects. Fortunately, the group had technical and production help from Bjarne Gram Hansen, postdoctoral scientist (see p. 12: Solving the secrets of drug genes); Dorte Holm, PhD student with Mortensen and Professor Jens Christian Frisvad; and student Katrine Husted Brogaard.

The next step of the project takes advantage of new resources at CMB (see p.16: New infrastructure, new potential). "From the beginning we looked for methods that could be automated," says Lynge Nielsen, and Holm will take advantage of this strategy. She will be using the new high-throughput robotic system to generate strains that overexpress polyketide synthesis pathway genes. The group can also begin exploring genes encoding enzymes that tailor and modify the basic polyketide structure, such as by adding sugars. These generate the broad diversity of molecules in the polyketide class and are the most interesting for learning about polyketide-based toxins and drugs. "The central genes make the main scaffold molecule," says Lynge Nielsen. "But the money is in the tailoring enzymes that make the final products." These new investigative directions are supported by the upgraded equipment of the analytical platform, which Klejnstrup says should allow the chemical analysis team to detect and isolate new compounds that were missed before.

So far, the project has identified polyketide genes as expected, and yielded surprises with the complicated chemical profiles of the fungal deletion strains. "It's been fun and everybody has learned a lot, "says Lynge Nielsen. "Knocking out the genes gave us a global view of polyketides and told us that it's more complex than we expected. In addition", he says, "This has brought the centre closer together because it's the first time we've done this kind of multidisciplinary project – the first large-scale project with chemists and molecular biologists and mycologists all working together."



A global hunt for mari ne-derived antibiotics

By Chris Tachibana for CMB

A PhD project goes from old-fashioned exploration biology to state-of-the-art chemical analysis

In 2007, the global research expedition Galathea 3 returned to Copenhagen after a nine-month tour on the ship Vædderen. Researchers on board collected hundreds of bacterial samples from oceans around the world, hoping to solve our growing antibiotic resistance problem. In 2011, CMB student Maria Månsson defended her PhD thesis, "Discovery of Bioactive Natural Products from Marine Bacteria", showing this might be possible.

More than 500 of the bacterial strains collected on the Galathea 3 expedition by Professor Lone Gram and co-workers from DTU Food and CMB inhibited pathogenic bacteria. Månsson focused on strains from the genera *Pseudoalteromonas* and *Vibrio*, hoping to identify compounds that might be new antibiotic therapies. First, she needed to evaluate the large collection of bacteria for their potential to produce known and new antibiotics. This required establishing new, efficient methods that bridged biology expertise at DTU Food with chemistry knowledge at CMB. Månsson and her collaborators had to figure out growth conditions that made the bacteria produce antibiotics, and then link that production to a specific chemical profile.

“We found that one way to unlock new chemistry was to mimic the natural conditions of the bacteria,” says Månsson. One success was supplementing the growth medium of the marine bacteria with chitin, a carbohydrate found in seawater because it is a major component of plankton exoskeletons. Other techniques also had to be tweaked. "Marine bacteria need sea salt to grow, and for a chemist, salt is a big challenge," says Månsson. Too much can affect downstream chemical analysis, like the chromatography and mass spectrometry steps. "A lot of my time in the project just dealt with low-tech challenges," she says, such as altering established CMB protocols for extracting compounds from fungi that did not work with the marine bacteria.

CMB expertise, infrastructure, and collaborations, along with Månsson's hard work, has been crucial to the project. Månsson and her collaborators first prioritized the bacterial strains, finding those with the greatest potential to produce antibiotics. The first step in this process was a chemical analysis screen to map all compounds from the different strains, because bacteria often produce multiple bioactive products. Also, unrelated bacteria, or bacteria from different locations can produce the same compounds. One particularly interesting strain from the Galathea expedition was from the surface of a mussel found near the Solomon Islands off Australia, but exotic as that sounds, a soil bacterium from a Danish garden might produce exactly the same antibiotic. "We needed to do careful dereplication," says Månsson, "which is excluding compounds that are already known. People have been screening for antibiotics for a long time so it happens a lot that you find something already known, but it is still worth doing to solve the antibiotic resistance problem."

The project has an edge in discovering new compounds, because few researchers screen for antibiotics from marine bacteria. Filamentous soil bacteria are considered more interesting because they have already yielded antibiotics such as streptomycin. However, Månsson says, "By optimizing all the purification steps for the best possible yield, and with more sensitive measurements, we can look at more of the natural products that bacteria make." The good news for potential drug developers is that marine bacteria may produce especially potent candidate drugs. "When bacteria in the ocean secrete compounds," says Månsson, "they are diluted in the sea, so the compounds usually have a lot of activity in small amounts." These small quantities can be detected and characterized thanks to the highly sensitive analytic equipment at CMB, and access to the powerful magnetic resonance imaging facilities at Carlsberg Research Laboratories.

Månsson named the first two compounds she discovered solonamides. These are small, cyclic peptides that contain unusual amino acids. They do not kill pathogenic bacteria, but quench their virulence. Collaborating with researchers at KU-LIFE, Månsson found that two different solonamides reduced the virulence of USA300, a community-acquired strain of MRSA−methicillin-resistant *Staphylococcus aureus*, that is infamous for infecting young, healthy people. The solonamides are fascinating, but "we don't know how they work," says Månsson. "They resemble molecules used by *S. aureus* to regulate their own virulence so we suspect they are competitive inhibitors that block communication receptors." Virulence factors that might be affected include secreted toxins, or tissue-degrading enzymes. Before solonamides show up in the pharmacy, however, their potential toxicity towards mammals must be explored. Månsson's advisor Associate Professor Thomas Ostenfeld Larsen says, "This project has given us an excellent chance for close collaborations with DTU Food, the University of Copenhagen and other partners, and we hope to expand our marine chemistry activities in the coming years."

Månsson hopes the project will launch an academic career in natural product chemistry. "I'm excited about the ecology of these compounds, to try to understand why they are produced, and the biological systems they affect. It's fascinating to dig into a microniche and find the hundreds of bacteria associated with a single algae or marine animal and see how they affect each other with their plethora of chemical substances." Månsson will spend the year of remaining project funding applying her methods to more strains in the Galathea collection, saying, "I focused on 5 strains, but we have 500."

More water-repellent than Teflon

By Chris Tachibana for CMB

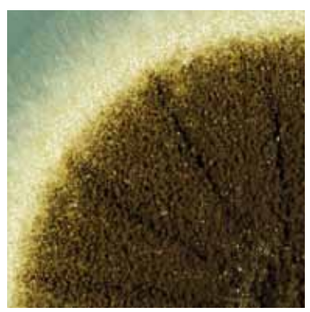
Water-resistant proteins that coat fungal cells might be a target for antifungal vaccines

Imagine a drop of water on Teflon. It beads up and rolls right off. This does not impress Mona Højgaard Pedersen. She is studying small fungal proteins called hydrophobins that "are even more water-repellent than Teflon," for her postdoctoral project with CMB Professor Jens Christian Frisvad. Frisvad explains that a coat of hydrophobins keeps rain from soaking into forest mushrooms. Hydrophobins keep the spores of filament-forming fungi dry, and help them become airborne. For spores of *Aspergillus fumigatus*, the layer of hydrophobins also acts as a protective shield, increasing the pathogenicity of this common fungus. Healthy people can combat the spores, but immune-compromised patients are susceptible. Mortality rates for an invasive *Aspergillus* infection are reported to be 30-95%.

"We wanted to characterize hydrophobins from this species because it is a human pathogen," says Pedersen. *A. fumigatus* has genes for five hydrophobins, and Pedersen has been working on two called RodA and RodB. RodA forms the shield on fungal spores, but Pedersen says, "RodA is shed as the fungus grows, so it's a poor vaccine target." She has focused on RodB, because "it is expressed when the fungus forms a biofilm, which is a prerequisite for infection." Cells that stick together in a biofilm are protected from drugs and from the immune system, so attacking this defence mechanism could weaken an invading fungus. To get a good biochemical look at both hydrophobin proteins, Pedersen needed to isolate substantial amounts of each. RodA was fairly easy, and could be purified from cultures of *A. fumigatus*. RodB was much less cooperative. "I couldn't find conditions to get RodB," says Pedersen, "and anyway I had to use a harsh extraction method for both of them because they are not water-soluble." The results were not satisfactory, with most of the proteins damaged and fragmented.

"We were saved by molecular biology and fermentation," says Pedersen. "It would have been completely impossible to get RodB without it." With Assistant Professor Irina Borodina, Pedersen cloned the genes for RodA and for RodB and put them into the yeast *Pichia pastoris*. Frisvad explains that filamentous fungi like *Aspergillus* produce hydrophobins, but single-celled yeast like *Pichia* do not. However, as a fungus, *P. pastoris* has the cellular equipment to make and properly fold hydrophobins. Since it doesn't make them itself, any hydrophobins the engineered strain makes are the *A. fumigatus* type. Using CMB cell engineering and fermentation expertise and a lot of trial and error, Pedersen generated enough protein for analysis – up to 329 mg of hydrophobins per litre of culture. She also engineered the hydrophobins to be secreted into the culture medium for easier isolation. To each hydrophobin, she added a signal sequence, which is a short segment of amino acids that directs a protein to a specific location, like outside the cell. "We didn't know

if they could be secreted in water-soluble form but they could, if the signal sequence was added," she says. The unusual chemical properties of the proteins make them tend to stick together, complicating purification. "We lost 90% but we got so much to start with that we had enough to do basic research," says Pedersen. With postdoctoral scientist Jacob Lange Moresco and Assistant Professor Winnie Edith Svendsen of DTU Nanotech, Pedersen showed that both RodA and RodB from *A. fumigatus* have the characteristics of other fungal hydrophobins, like the ability to form a hydrophobic coating on a surface. The group is also working to get three-dimensional structures of the hydrophobins with Johan Olsen in Birthe Kragelund's lab at the University of Copenhagen. The results should be interesting to the fungal and protein structure communities, says Pedersen, because, "There are hundreds or thousands of hydrophobins and only three have a solved structure, and they are not from *Aspergillus*. RodB has been looked at only on a genetic level, so far. As far as we know, we are the first to have it in a tube to investigate."



The project is also going in a genetic direction, with PhD student Britt Guillaume Jensen knocking out hydrophobin genes in the less pathogenic *A. nidulans*. Pedersen says, "We're interested in 'the big why', what are they good for?" In other words, what does the fungus use them for? Frisvad say that another area of interest is the coexistence of *A. fumigatus* and the bacterium *Pseudomonas* in the lungs of people with cystic fibrosis. A possible project is exploring if these two opportunistic pathogens remain in separate foci in infected lungs, or if they make a biofilm together, and if so, if hydrophobins are involved.

Pedersen has nine months left in her postdoctoral project, so she is generating antibodies against hydrophobins and will use them to see if RodB is continuously present on the fungal surface, which would make it a potential target for a drug or vaccine. "We'll use the antibodies with a fluorescent tag to see if we find it while the fungus is growing. If it is present at all times, especially when the fungus is actively growing, that would make it a excellent target," says Pedersen. Her work so far has just been published in *Applied Microbiology and Biotechnology.*

Solving the secrets of drug genes

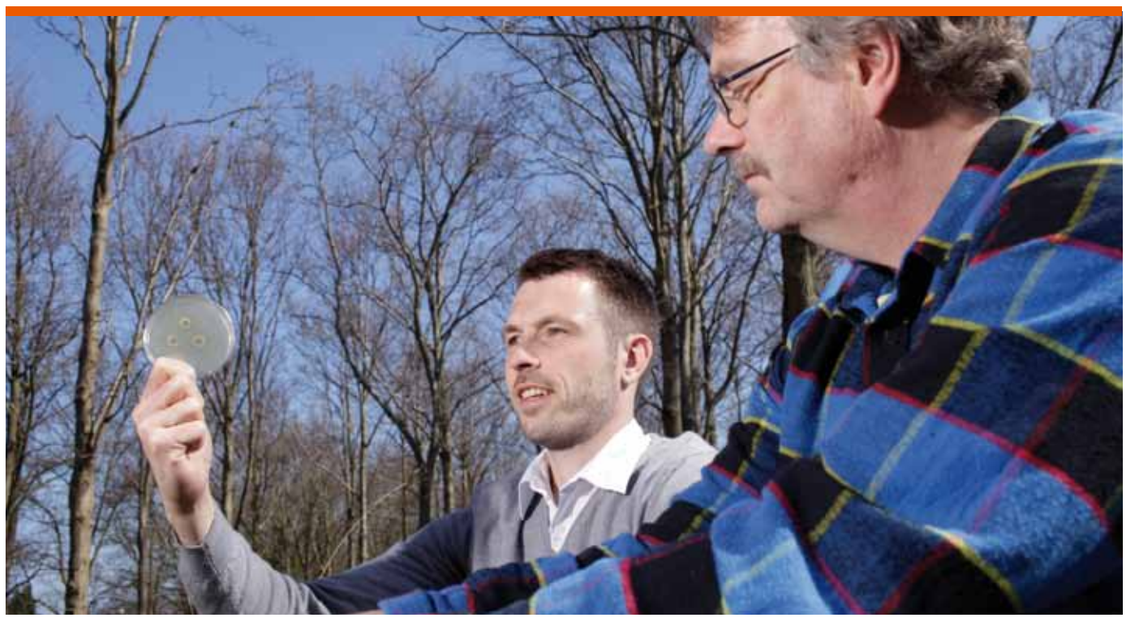
By Chris Tachibana for CMB

It all started when Torsten Regueira, then a CMB PhD student, discovered a treasure of genes lined up on a fungal chromosome. The gene cluster looked like the key to producing mycophenolic acid (MPA), the active ingredient in a number of drugs used to prevent organ transplant rejection. In fact, the genes were from *Penicillium brevicompactum*, one of the few species that make MPA. But appearances can be deceiving, so Bjarne Gram Hansen set out to study the genes more closely in a postdoctoral project with Associate Professor Uffe Mortensen and Assistant Professor Kiran Patil, now at the European Molecular Biology Laboratory in Heidelberg. Professor Jens Christian Frisvad contributed expert advice on fungal strains and traits. "One goal was expressing the MPA cluster in a heterologous host," says Hansen. That is, transferring the MPA biosynthesis genes to another organism that does not make MPA, in this case the filamentous fungus *Aspergillus nidulans*. If the engineered fungi made MPA pathway compounds, Hansen would have solid evidence that he was working with the MPA gene cluster.

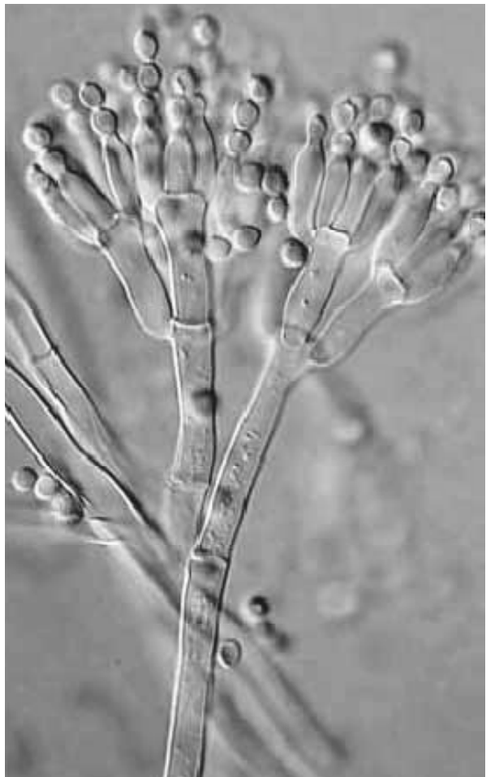
The experiment also tested CMB's toolbox (see CMB 2009 Annual Report), a set of fungal strains and genetic hardware for removing, adding, changing, or measuring the expression of genes in those strains. To the toolbox, Hansen added a set of plasmids, small DNA molecules useful for shuttling genes between different cell types, and used the tools to put MPA pathway genes into *A. nidulans*. The genes were too large for standard genetic techniques, so Hansen introduced new, more efficient genetic manipulation methods to the CMB toolbox. "There was no way to do it with old-fashioned cloning using restriction enzymes and ligase," says Hansen. Dorte Holm (see p. 6: Attention!... Calling all competencies), a Master's student with Hansen who is now doing a PhD project with Mortensen and Frisvad, rapidly isolated 40 very large polyketide synthase genes using the method, an accomplishment that would have been an entire PhD thesis a few years ago. CMB is the optimal place for this project, says Hansen, because the facilities are in place to "go all the way from the tools in filamentous fungi to chemical analysis of the products."

A. nidulans was the perfect fungus in which to test the gene cluster. It does not make MPA, but MPA is a polyketide and *A. nidulans* makes many of those, so it has the basic cellular equipment to produce MPA. The first *P. brevicompactum* gene Hansen added to *A. nidulans* was for the polyketide synthase enzyme that catalyzes the first step in MPA synthesis. The engineered cells happily produced the first pathway intermediate. "When the polyketide was synthesized, we were pretty sure we had the right cluster," says Hansen. "Also, this is the most difficult step in MPA synthesis, so it should be easy to do the rest, and make MPA."

But first, *A. nidulans* must be made MPA-resistant. MPA targets the enzyme inosine-5'-monophosphate dehydrogenase (IMPDH). MPA prevents organ transplant rejection because immune cells that attack foreign tissue rely heavily on this enzyme, and are particularly sensitive to MPA. However, MPA is generally toxic. "Basically, all eukaryotes are sensitive to it," says Hansen, except, of course, the *Penicillium* species that make it. Why aren't they affected by MPA? "We were very interested in the resistance mechanism," says Hansen. One gene in the



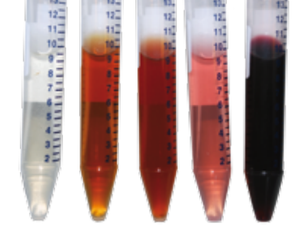
cluster encoded an extra IMPDH enzyme, and transferring it into *A. nidulans* converted it from MPA-sensitive to MPA-resistant. Part of the resistance is just having the extra gene and making more IMPDH enzyme, but the enzyme is also special. "We can't kill it with MPA," says Hansen.



After the polyketide synthase and resistance genes were identified, the MPA cluster yielded an unexpected discovery. Repeated computer analysis of the cluster suggested that two adjacent genes, predicted to encode two very different enzymes, were actually just one gene. Eventually, Hansen left the computer and went into the lab to test if there were one or two genes. He found that instead of two different RNA transcripts, which would have been the result if the two genes were separate, only one transcript was made, showing that the two genes were fused. The fusion enzyme was predicted to work at the second step in MPA synthesis, so Hansen put the gene into the *A. nidulans* strain producing the first MPA intermediate. The single gene was enough to catalyze two reactions. Although some enzymes have two activities, this is two different, full-length enzymes in a single protein – one a cytochrome P450 and the other a hydrolase. "I realized after I found out it was a fusion enzyme that fusion enzymes are very rare," says Hansen. "This is two different biosynthetic enzymes that just by chance ended up as one enzyme."

Hansen's success with the MPA gene cluster has led to a job as a research scientist with Novozymes, but he leaves behind at least one mystery to be solved – why the IMPDH enzyme from the *P. brevicompactum* cluster is so MPA-resistant. Mortensen says, "We're collaborating with Professor Lisbeth Hedstrom at Brandeis University in Boston, who's an IMPDH expert, to find out just what makes this enzyme so resistant." Stay tuned, because the MPA gene cluster may have more surprises in store.

Mould toxins from field to flask



By Chris Tachibana for CMB

MB analytical chemistry prowess detects mycotoxins in crops and in the supermarket

CMB PhD student Jesper Mølgaard Mogensen might approve of the general idea expressed in the Omar Khayyám quote, "A loaf of bread, a jug of wine, and thou"– but Mogensen would take a close look at that bread and wine. His research shows that fumonisins, which are carcinogens made by certain moulds, are surprisingly common in maize, and in grape products like wine.

Mogensen's project was prompted by an unexpected discovery. When the genome of the common black bread mould *Aspergillus niger* was sequenced in 2007, it was found to have the genes to produce fumonisins. CMB scientists and others showed that *A. niger* actually produces a number of different fumonisins. Previously, these fungal toxins, or mycotoxins, were known only from moulds in the *Fusarium* genus. "Scientists around the world are still surprised that *A. niger* is able to produce fumonisins," says Mogensen. The discovery is not just a scientific curiosity. Fumonisins are implicated in cancer and other diseases in humans and animals, so Mogensen's project is "to investigate whether fumonisins produced by *A. niger* are an overlooked health risk in Denmark as well as worldwide." His research with advisors Associate Professor Kristian Fog Nielsen, Associate Professor Thomas Ostenfeld Larsen and Professor Jens Christian Frisvad, is funded for three years to survey "the occurrence and levels of fumonisins in various raw materials, food products and possibly ingredients produced by biotechnological processes from a variety of countries." The project has a broad scope because *A. niger* is commonly used in industrial production of food additives, and grows on many foods.

A. niger is reported to be found on 80% of grape samples, which could explain why CMB PhD student Peter Boldsen Knudsen and Mogensen found that half of retail raisin samples had detectable fumonisins. The team analysed 21 brands of raisins from six countries, from markets in Denmark, Germany and the Netherlands. Regulatory agencies don't specify an acceptable amount of fumonisins in raisins, but the amounts Knudsen and Mogensen found were far below allowable levels in Europe and the US for other foods, such as maize.

Moving to grownup grape products, Mogensen surveyed 77 bottles of wine from 13 countries. He detected fumonisin in 28% of the reds, 7% of the whites and in one bottle of port. Again, levels were generally low, at 1-25 µg/litre, although no regulatory standards exist for fumonisins in wine. "The frequency was the unexpected result," says Larsen. "We don't know the effects of long-term fumonisin exposure, such as from drinking wine over many years, but our collaborators at DTU Food will be looking into the toxicology and health risks." Mogensen's poster on detection of fumonisins in grapes, raisins and wine won a prize at MycoRed Africa 2011, the conference of an international consortium funded by the European Union to reduce mycotoxins in the feed and food chain.

Beyond finding that fumonisins are more common in foods than previously thought, the project has created new methodologies for detecting mycotoxins in food samples. This is important for future studies, because using consistent methods allows results from different studies to be compared.

Future studies should go more quickly, with the new instrumentation for compound separation and detection in CMB's Analytical Platform (see p. 16: New infrastructure, new potential). "Analysis is more sensitive, and about 2.5 times faster," says Nielsen. As Mogensen's main PhD project advisor, he may expect at least 2.5 times more results with the new equipment, and he might get it. The platform was essential for Mogensen's latest results, analysing maize from farmers in the former Transkei region in southeast South Africa, where fumonisins are implicated in the high rate of esophageal cancer. The farmers had sorted maize kernels into good quality, used for human consumption, and mouldy, used to brew beer. Mogensen found that the farmers' sorting technique was generally effective: the good-quality batches had 0.28-1.1 mg/kg fumonisin, and the low-quality batches had 0.03-6.2 mg/kg. However, the extraordinary sensitivity of the new analytical platform meant that individual kernels could be sampled, giving an analysis that can only be called finely grained. Mogensen found that when a batch of maize had a high average fumonisin content, usually a few highly contaminated kernels were responsible. His conclusion was that more careful sorting could reduce the total fumonisin content in the maize batches by 71%. This is exactly the kind of low-tech, low-cost solution to mycotoxin contamination that MycoRed hopes to find.

Mogensen will complete his project in late 2011 and likely defend his PhD in 2012. The international feature of his work on food and wine has had an effect. He hopes to get funding to continue work as a postdoctoral fellow at CMB, "combined with an external stay at another university." Who knows where Mogensen will end up, but South Africa has fine academic institutions, plenty of maize samples, and a growing wine industry.

New infrastructure, new potential

CMB capabilities expand with new fermentation, mammalian culture and analytical platforms, and a high-throughput robot

Scientists want more, and they want it faster and better. Streamlined, accurate processing of multiple samples extends a researcher's investigative power. Recent additions to CMB infrastructure increase scientific capacity, capability, and speed.

Fermentation

The new CMB facilities begin with the heart of industrial biotechnology: fermentation, or culturing microorganisms to generate a product. CMB is now the home of the Department of Systems Biology Fermentation Platform, thanks to 30 million DKK from DTU. CMB hosts the facilities, with Associate Professor Jette Thykær as its leader, because of the centre's fermentation expertise. "This doubled our fermentation capacity, but more than doubled our research capability," says Thykær. "We now do fermentations in parallel that we used to do sequentially, and these resources give us an advantage in grant applications." DTU students benefit through opportunities for hands-on experience. Standardized software makes it easy to scale through the 0.5-, 1-, 2-, 5- and 10-liter systems.

Supporting 63 bioreactors are laminar flow benches, shaking incubators, autoclaves, and the flashiest component, a mass spectrometry off-gas detection system. Tubes from up to 60 bioreactors lead to a central analyzer that tracks individual culture gases. "With this instrument, we have a precise and reliable measurement of the oxygen and carbon dioxide levels to monitor culture growth and health," says Thykær. Other volatiles such as alcohols can also be detected.

Mammalian cell culture lab

The platform funds set aside 1.6 million DKK for mammalian cell culture facilities, so "We've been shopping like maniacs," says Mikael Rørdam Andersen who started the project as a post doc but recently made Associate Professor. He and post doc Helene Faustrup Kildegaard, both with Associate Professor Uffe Mortensen, are in charge, and are making the most of the funds. Two culture rooms contain incubators, bioreactors, a cell counter, a microscope, two bioanalyzers for monitoring cells, a laminar flow bench, a fume hood, and equipment for processing and analyzing cells. Mammalian cultures are notoriously finicky and easily contaminated, so everything is for mammalian cells only. Culturing started in December 2010 with Chinese hamster ovary (CHO) cells, a research and industry standard, with plans to expand to additional lines. The first project is Kildegaard's – investigating yield from antibody-producing cells.

"We're the only university in Denmark offering training in industrial mammalian cell culturing," says Andersen, so the labs are a unique opportunity to learn techniques common at companies. "A lot of students are interested in mammalian cell projects," says Kildegaard, and several now work on collaborative projects with other DTU departments, Symphogen, and Novo Nordisk. This is the first year that a CMB course on heterologous protein production in mammalian cells will be on-site instead of at Bioneer A/S. Upcoming projects include using CMB's high-throughput facilities to generate new vectors for genetic engineering of mammalian cell lines.

High-throughput facility

High-throughput work is now possible at CMB with a Hamilton Microlab Star robot. Post doc Irina Borodina set up the system and says "it's the absolute best." The robot is an assembly line of components that process microtitre plates – rectangles with wells for 96 samples. Components were chosen for versatility, says Borodina. Plates can be heated or cooled; transported to a reader that detects fluorescence, absorbance, or luminescence; or shuttled to an incubator for cell culturing. A vacuum system allows DNA or protein isolation. Unlike humans, the robot follows directions exactly, and can be programmed to retry a problematic step, or send an SMS to the researcher and await instructions.

To analyse DNA or protein in complex samples such as PCR reactions or fermentation broth, a Caliper LabChip GXII microfluidics electrophoresis system automatically processes microtitre plate samples, taking about 30 seconds per sample. Information about the wells with the best samples for the next experimental step can be transferred to the robot.

Borodina shows off the first tests: 20 yeast strains cultured in four media in triplicate, with growth curve points every 30 minutes. Future uses include PCR reactions, cloning, DNA purification, cell transformations, and enzyme activity assays, all for hundreds of samples. A protocol is in development to grow fungal strains and perform chemical extractions in a single microtitre plate.

Analytical abilities

Analysing and identifying compounds in a microbial extract, feed or food sample is standard practice at CMB. The technique is used to characterise fungi by chemical profile (see p 6: Attention!... Calling all competencies) to deduce biosynthetic pathways and metabolic regulation, and to screen bacteria for antibiotics (see p 8: A global hunt for marine-derived antibiotics). Now, the centre has new equipment to meet increasing analytical demands and challenges.

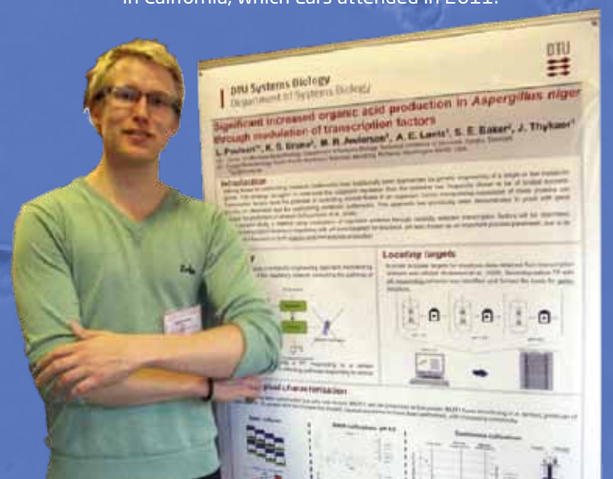
Associate Professor Kristian Fog Nielsen is in charge of the new instruments, purchased with national research equipment funds and operating since October 2010. He says they are "basically the same as our older equipment, except the data quality is tremendously better, meaning that we can solve problems much faster. Also, we can see 10- to 100-fold lower amounts." The analytical platform has new ultra high-performance liquid chromatography and ion-chromatography equipment for enhanced separation performance, which also boosts the mass spectrometry capabilities. Recently, the platform's power was demonstrated by detection of fungal toxins in single maize kernels (see p 14: Mould toxins from field to flask). The new platform also tripled the number of polyketide intermediates detected in *Aspergillus nidulans* mutant extracts. The infrastructure additions give new power to CMB researchers, and provide students with access to state-of-the-art facilities. Of the analytical platform, Nielsen says the instrumentation is "as good as the best in industry, and better than in many companies."

CMB Annual Report

Highlights 2010

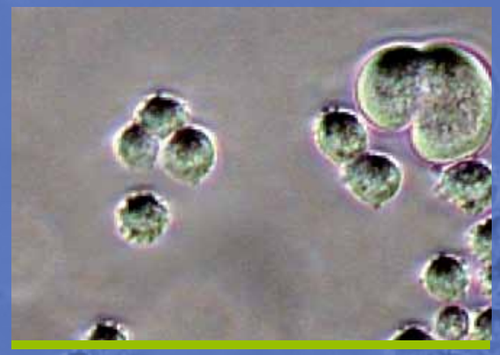
Renovations of CMB building completed Building 223, one of the two building hosting CMB at the Technical University of Denmark, saw the last of the renovations which were on-going since the summer of 2009. This means new lab facilities, office spaces and flexible meeting rooms for both work and social activities in the building. This a benefit for both students and employees who all worked hard to make both renovations and research come through.

Poster awards for PhD student Lars Poulsen PhD student Lars Poulsen won two poster awards in 2010. At the 5th Danish Conference on Molecular Biology and Biotechnology: Biofuels and Biorefineries, Lars won DKK 2000 for his poster "Modulation of transcription factors: A metabolic engineering strategy for improving *Aspergillus niger* as a production host", while his poster "Significant increased organic acid production in *Aspergillus niger* through modulation of transcription factors" won the poster award at the DTU Society for Biological Engineering (SBE) Symposium for Biotechnology Research. The prize was a trip to SBE's international conference on Biomolecular Engineering in California, which Lars attended in 2011.



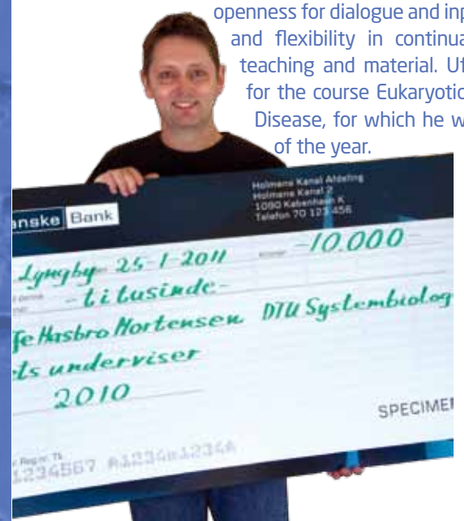
Elite post-doc scholarship Rasmus John Normand Frandsen joined CMB as a postdoctoral fellow in 2010. Rasmus completed his PhD "A study of Polyketide biosynthesis in *Fusarium graminearum*" at Copenhagen University, Faculty of Life Sciences, in 2010. Rasmus works on the project Chimeric iterative polyketide synthases – Technological platform for the identification and production of novel anti-bacterial compounds, which is an individual post doc grant by FTP, under Associate Professor Uffe Hasbro Mortensen. For this project, and in recognition of his outstanding previous work, Rasmus was named Ung Eliteforsker (Young Elite Scientist) by the Danish Agency for Science, Technology and Innovation. The award included a grant of DKK 200.000.

First Mammalian cell culturing at CMB One of the new laboratories in building 223 is specially approved for the work with genetically modified organisms. Post docs Mikael Rørdam Andersen and Helene Faustrup Kildegaard have been working with projects in this area, and has in 2011 opened the lab to students interested in the field. In 2010 CMB cultured the first mammalian cells in the labs; Chinese Hamster Ovary (CHO) cells.



New permanent member of CMB faculty Jette Thykær joined the CMB faculty in a permanent position as Associate Professor. Jette has been a part of CMB for 10 years, doing both her PhD and post doctoral fellowship here. Jette specialises in fermentation technology with focus on research within quantitative physiology of filamentous fungi, where she is a capacity for both CMB and the departmental fermentation platform which she heads.

Uffe Hasbro Mortensen is DTU Systems Biology teacher of the year 2010 Associate Professor Uffe Hasbro Mortensen was nominated the best teacher of the year at the department of Systems Biology. The students at the department award the prize to one of the department's 50 teachers. When presenting the award the students praised Uffe's innovation in teaching, openness for dialogue and input from the class and flexibility in continually adapting his teaching and material. Uffe is responsible for the course Eukaryotic Cell Biology and Disease, for which he was made teacher of the year.



Mycotoxin workshop hosted at CMB In June 2010 CMB Associate professors Birgitte Andersen and Kristian Fog Nielsen hosted the 32nd workshop of The Society for Mycotoxin Research. More than 200 delegates from 25 countries attended the conference. The three-day programme had more than 150 scientific presentations within all areas of research in mycotoxins and fungal products, especially analytical methods and toxicology.

New mass spectrometer-equipment As part of the analytical platform at CMB, Associate Professor Kristian Fog Nielsen purchased a new MS: Bruker maXis G3, quadrupole time of flight mass spectrometer, for sub ppm accurate mass determination, fitted to a UHPLC system for ultra fast separations. It arrived in autumn 2010, and its dimensions made installing it an exercise which was successfully navigated. The MS has been functioning since October 2010.

Faculty

Associate Professor
Birgitte
Andersen

Professor
Jens Chr.
Frisvad

Associate Professor
Timothy
Hobley

Assistant Professor
Kaisa
Karhumaa

Professor
Morten C.
Kielland-Brandt

Associate Professor
Anna
Eliasson Lantz

Associate Professor
Thomas
Ostenfeld Larsen

Associate Professor
Uffe
Hasbro Mortensen

Associate Professor
Kristian
Fog Nielsen

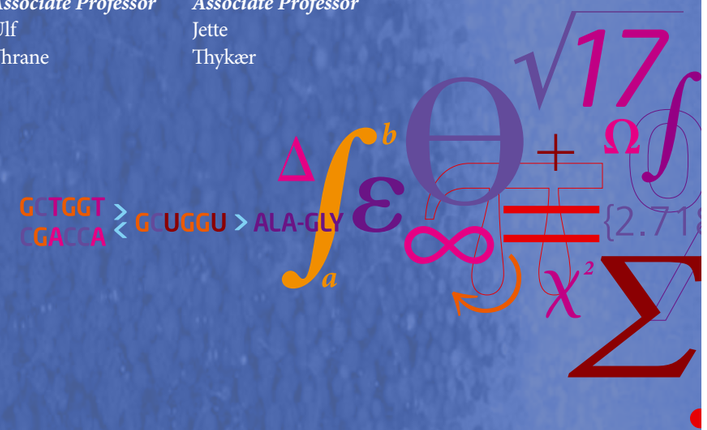
Associate Professor
Michael
Lynge Nielsen

Assistant Professor
Kiran
Patil

Associate Professor
Ib
Søndergaard

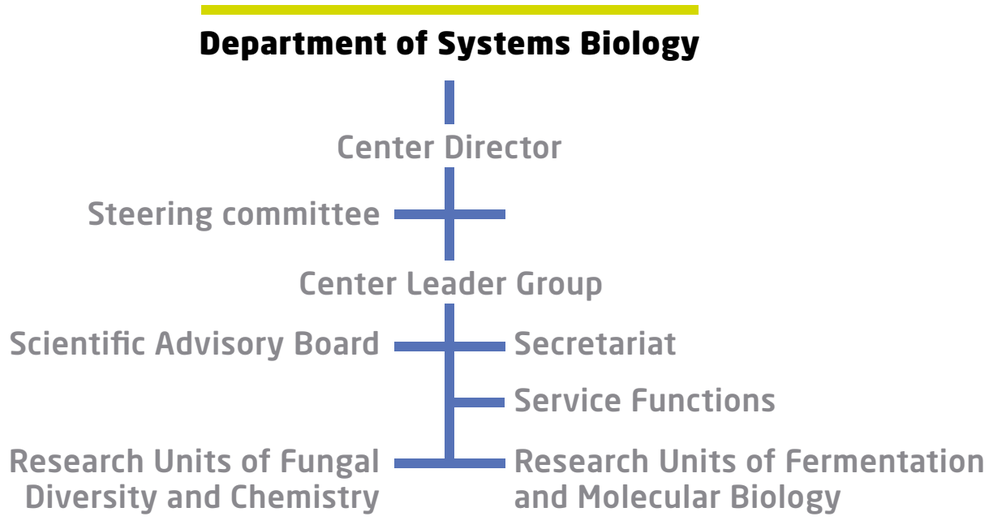
Associate Professor
Ulf
Thrane

Associate Professor
Jette
Thykær



Organization

The research at CMB is headed by our faculty members, and is organised in research groups. Each group leader delineates the strategy of the research group in accordance with the overall mission and vision of CMB as defined by the Center Director. The management group is selected by the Center Director amongst faculty.



The Scientific Advisory Board comprises five internationally recognised researchers in fields covering the majority of the research activities at CMB. The advisory board meets in connection with CMB's annual meeting, and assists in the evaluation of research activities.

Professor Axel Brakhage,
University of Hannover, D

Professor Bärbel Hahn-Hägerdal,
Lund University, SE

Professor Carsten Christophersen,
University of Copenhagen, DK

Professor Jack Pronk,
Technical University of Delft, NL

Professor Peter Roepstorff,
University of Southern Denmark, DK

Steering committee provides strategic and managerial advice and approves significant decisions, e.g. the approval of new long-term scientific positions. The board meets with the Center director twice a year, and once a year writes a brief statement about the center for the Danish Research Agency.

Professor Klaus Bock
Chairman of the Danish National Research Foundation

Director of Research Jørgen Hansen
CEO of Evolva Biotech A/S

Senior Director Carsten Hjort
Novozymes A/S

Professor Søren Molin
Technical University of Denmark

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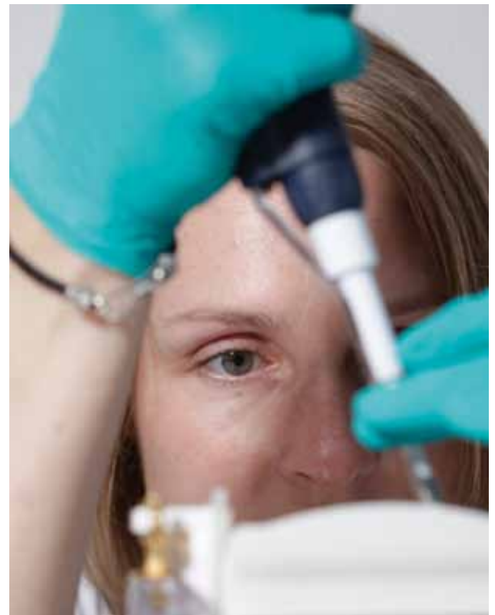

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Jayavelu, Tamilselvan, Guest researcher
Manikowska, Joanna (Asia), Guest student
Montagud, Arnau, PhD student
Subramanian, Ramalingam, Guest researcher

The petri dish shows the growth of *Aspergillus niger* from the naturally occurring spores on raisins left for one week on a growth medium.

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