Annual Report **2008**

Center for Microbial Biotechnology
Department of Systems Biology

Center for Microbial Biotechnology
Department of Systems Biology
Technical University of Denmark
Building 221 / 227
DK-2800 Kgs. Lyngby
Denmark

Phone: +45 4525 2600
Fax: +45 4588 4148
doan@bio.dtu.dk
www.cmb.dtu.dk

Articles written by: Chris Tachibana
Edited by: CMB

CMB is an Engineering Center of Excellence funded by the Danish Research Agency. It is a collaboration between an acknowledged research manager, his/her institute and university, and the Research Agency. An Engineering Center of Excellence is a research institute of first-class quality with tradition for cooperation with industry.

DTU Technical University of Denmark

Contents

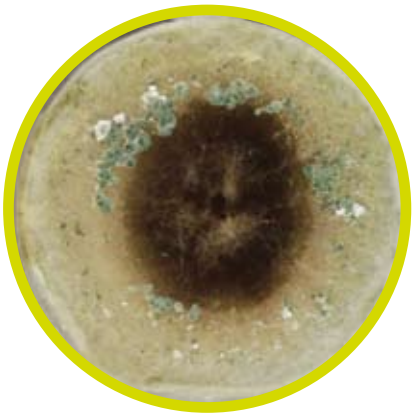
Communication and cooperation between mould species permits better growth for all

CMB Associate Professor Birgitte Andersen investigates how chemical communication between fungal species may allow moulds to cooperate and thrive on water-damaged surfaces like wallpaper, wood and fabric.

By Chris Tachibana

Step one: keep out the water. This is the key to preventing mould from growing on your windowsills, walls and furniture, says Associate Professor Birgitte Andersen. A decade ago she studied fungal growth on building materials as part of the *Moulds in Buildings* project, a collaboration of doctors, mycologists, and builders that looked at the health impact of mould in water-damaged schools, and came up with ways to renovate mouldy buildings. Andersen has continued work on building moulds, with funding from the Villum Kann Rasmussen Fund. She knows that step one is, in fact, impossible to fully implement, because of the humidity we generate in our homes when we cook, bathe and wash our clothes. Leaks and majors floods, like after Hurricane Katrina in New Orleans, can lead to extensive fungal growth. So step two is figuring out how to inhibit fungal growth, and this requires knowing exactly what nutrients fungi use on indoor surfaces and what their vulnerabilities are. Why not just live with the fungi? Microbial growth causes deterioration and discolouration when it breaks down building material. Some people are allergic to airborne mould spores, and in high concentrations, they can irritate the respiratory system. Some moulds produce mycotoxins that can cause illness, but toxin production is highly species-specific, making precise identification of fungal contaminants even more important. Although there are hundreds of thousands of fungal species, Andersen and colleagues have found that only a limited number grow indoors. Only 10-15 different types, such as *Penicillium, Aspergillus, Chaetomium, Stachybotrys* and the three that Andersen is currently concentrating on, *Cladosporium, Alternaria* and *Ulocladium*, are regularly found on building materials.

Some of these are the same taxonomic groups that attack food, so Andersen, with the help of undergraduate researchers and a technician, has used knowledge about food-spoiling fungi as the background for some hypotheses. For example, food-spoilage moulds are known to specialize, with particular species and strains adapting to growth in conditions like high salt or high protein. It appears that building moulds do the same, specializing to grow on wet wallpaper, wood or insulation material. Andersen has also found, however, that the building moulds tend to grow in consortia, or cooperative groups. In samples collected as part of the earlier project, Andersen found that two or three different species are usually found growing together. No single species dominates over the others, and all seem to grow more robustly than when each species is grown alone



Ulocladium (black) growing uninhibited together with *Trichoderma* (bluish green) Inoculated at the same time in the centre of a PCA plate.

Already established *Aspergillus* (3 orange cultures) "allows" *Trichoderma* (centre) to germinate and grow.

in pure culture. In addition, some unexpected patterns are seen, such as the appearance of *Stachybotrys,* which does not produce cellulase enzymes, on high-cellulose material. Association with cellulase-producing fungi, like *Ulocladium*, allows its growth. Other results suggest that the production of secondary metabolites influences the types of fungi in a consortium, with some compounds acting as mycotoxin defences for the entire consortium and others as deterrents for "outsider" fungi. These findings have led to the three main hypotheses of Andersen's current project: that mould on building materials is a consortium of collaborating fungal species; that each produces compounds such as enzymes or antibacterials that aid the consortium; and that members of the consortium communicate through compounds that stimulate or restrict other fungi.

The project is currently a basic research enterprise that uses species collected in the earlier study, grown on wallpaper samples prepared in the lab. The goals are to complete the identification of the fungi and characterize their individual limits to growth and metabolite production. Then, working with the fungal species in pairs and groups of three, Andersen and her colleagues will investigate which species are capable of creating a consortium, if and how they compete, and the small molecule communication or collaboration that occurs between them. Together with the Fungal Biodiversity Centre (CBS) in the Netherlands they have already used molecular analysis to definitively classify the moulds in some consortia, and have conducted preliminary metabolite analysis. They have found that different fungal species growing together on a Petri dish are not as competitive as one might expect, as long as they are grown on a medium that does not favour one or the other. Rather than one species taking over the culture, if the medium is poor for all of them and all are slightly stressed, they appear to equalize and possibly even collaborate, consistent with the hypotheses. This system could be a model for events that lead to fungal consortia "*in edificio*", on building materials that are acceptable, albeit poor growth media for several different collaborative fungi. In the future, Andersen will use HPLC-based metabolite mapping to identify the secondary metabolites that serve as communicating molecules, attractants, mycotoxins, anti-fungals and anti-bacterials in the consortia, possibly leading to targets for controlling indoor mould growth. For now, though, she advises we all try to follow step one and keep the humidity down and the water out.

Identifying Disease Biomarkers: A Networker's Approach

Assistant Professor Kiran Patil and his students are analysing existing transcriptome and metabolome data *in silico*, to find indicators for type 2 diabetes.

By Chris Tachibana

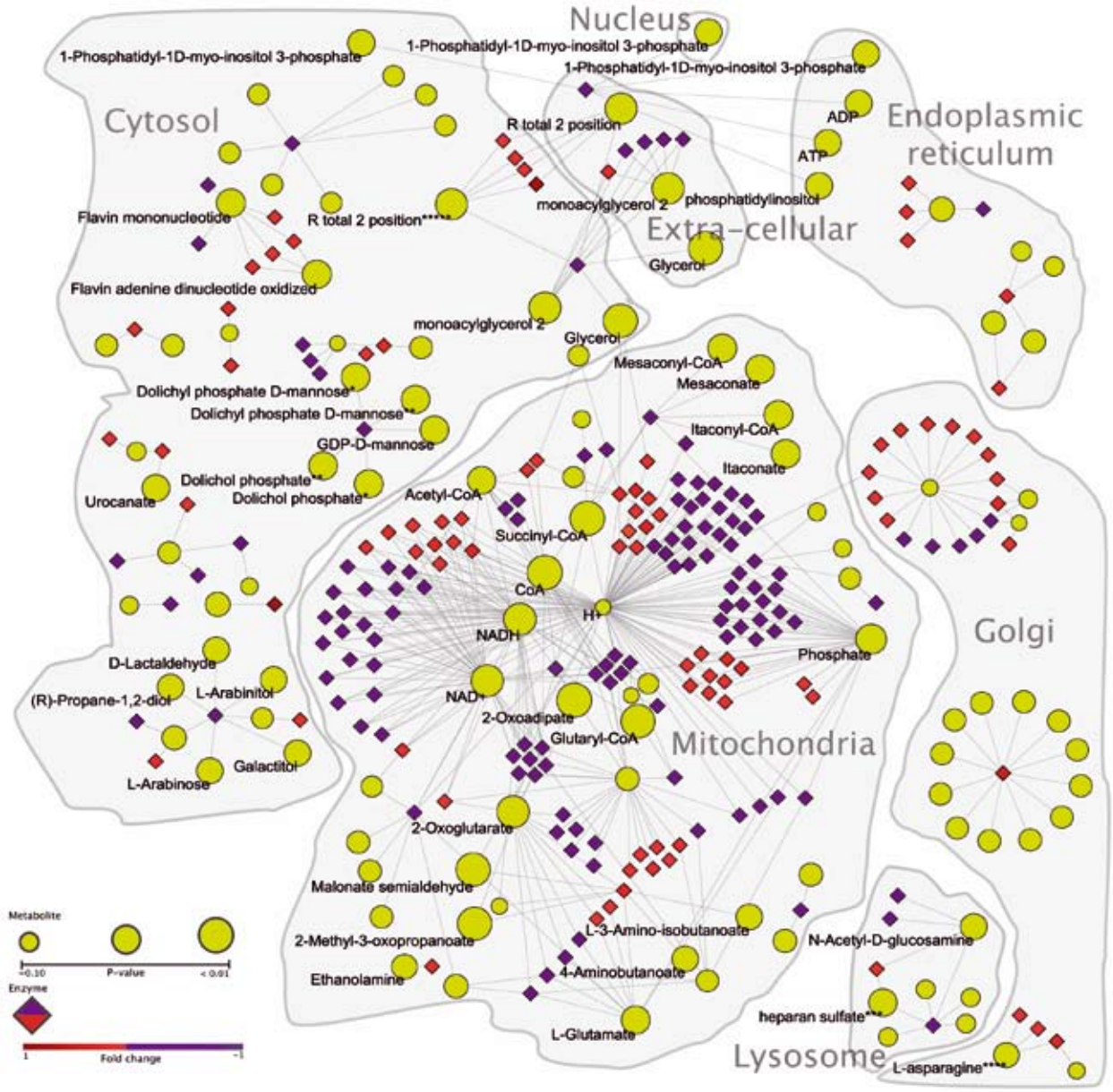
Kiran Patil is a networker. At CMB, he develops algorithms to analyze biological networks and their interactions. To find experienced personnel for his lab, he tapped into his professional network. He used connections in Portugal to recruit student Simão Soares, who brings expertise in informatics engineering. The lab now has a connection to Lithuania with Aleksej Zelezniak, who was recently awarded a prestigious Novo Nordisk-Novozymes Master's student scholarship. The team is working with collaborators Dr. Mary Elizabeth Patti at the Joslin Diabetes Centre in Boston, and Allan Ertmann Karlsen, from the Steno Diabetes Centre in Denmark, to untangle the complex interconnections between the cell's biochemical pathways, the metabolome of small molecules that flow through the pathways, and the transcriptome of the cell's expressed genes.

Their system for studying these network connections is type 2 diabetes, a disease of both insulin resistance and impaired insulin secretion that is linked to changes in metabolism, gene expression and the molecular composition of the cell. Type 2 diabetes currently affects millions of people worldwide and its incidence is increasing. In spite of its global importance, the causes, consequences and potential cures for type 2 diabetes are still being investigated. To make progress on these fronts, researchers and physicians need comprehensive information on what happens during the development of diabetes, and what happens if it is not treated. They need a fast, reliable and non-invasive diagnostic test, and they need a way to distinguish between different types of type 2 diabetes. For example, in Western countries, type 2 diabetes is often associated with obesity, while in Asian countries, it is not. In the absence of reliable markers to distinguish between forms of type 2 diabetes, little can be known about differences in their molecular mechanisms or consequences. Research on both diagnostic and distinguishing tests for type 2 diabetes would benefit greatly from the identification of biomarkers, or small molecules that act as indicators of the disease state and can be used to follow its progression. The diabetes work in the Patil lab is aimed at identifying these biomarkers. The hypothesis guiding the project is that the cellular objective in regulating metabolic genes is maintaining homeostasis. Rather than monitoring all possible small molecule changes at once, the cell relies on altering key metabolic fluxes, with all others following, as a way to maintain balance. The lab is looking for those key metabolites that are the main triggers of change.

In 2005 and 2008, the lab published papers describing Reporter Algorithms, a system that can be applied to genomic, transcriptomic, proteomic and metabolic data. The system uses information in public databases to make hypotheses about physiological and biological constraints that can be imposed on the vast amounts of data generated by global analysis. The method distills all possible interactions between pathways, gene expression patterns and metabolites, down to only the most likely and the most informative ones. Taking advantage of the microbial metabolomics expertise at CMB, the Reporter Algorithms system was first validated using established data on the yeast glucose repression transcriptome. Now, the methods can be applied to higher organisms. This has been demonstrated in plants, but Patil and his students have extrapolated their method to an even more complex system: the human diabetes transcriptome. Their initial analysis identified the TCA, oxidative phosphorylation and fatty acid metabolism pathways

as containing likely candidates for diabetes biomarkers. Promoter analysis of genes involved in the flux of these biomarker candidates led to the identification of the transcription factors CREB and USF1 as possible global regulators in type 2 diabetes, a finding that could be useful in identifying new drug targets.

Their next step was to apply the Reporter Algorithms to the available data on the human metabolome, and combine those results with the transcriptome data analysis. The results have led to powerful predictions for candidate diabetes biomarkers. Information on the candidates will be given to collaborators who have expertise in metabolomic analysis by mass spectroscopy, who will use human samples to test the hypotheses generated by the Patil lab. The collaborators will use the information on candidate biomarkers to focus their analysis on only those metabolites, out of the thousands of possibilities, that are the most likely to show a strong difference between the diabetic and non-diabetic state. In the meantime, the Patil lab continues to improve and extend the Reporter Algorithms, and apply them in making discoveries to guide basic research. An important and surprising finding is the importance of certain highly connected metabolites like NAD^+. This key metabolite occupies a node that connects the TCA, oxidative phosphorylation and fatty acid pathways, which appear to be crucial to metabolic flux in type 2 diabetes, and its importance helps explain the multiple effects of the disease. In a similar way, the researchers in the Patil lab also sit at a node, one that connects mathematical modelling with the interpretation and application of basic research, and the clinical development of diabetes tests and treatments.



Complementary competencies in a project to improve citric acid production

CMB researcher Jette Thykær travels to the United States to collaborate on increasing citric acid production from *Aspergillus*, and learns about Halloween and the Superbowl in the process.

By Chris Tachibana

To most people, *Aspergillus niger* is the mould on the sandwich left in the work refrigerator last week. But *A. niger* is also a factory, and it has two branches. One is represented by the strain of *A. niger* that is used for industrial protein production, for example, the amylase enzymes that are used commercially to make high-glucose and high-fructose syrups. Another strain is specialized for production of acid, particularly citric acid, which is widely used as a preservative and flavour additive in the food industry. CMB researchers have expertise in the protein-producing strain, and were involved in the project to sequence its genome. Researchers at Pacific Northwest National Laboratories (PNNL), which is funded by the United States Department of Energy, worked on the sequence of the acid-producing strain, so when both sequences were available, CMB and PNNL collaborated on a large-scale genome comparison. Taking advantage of this productive collaborative channel, and knowing that discussing results with colleagues over the phone or by email cannot compare to sitting together at a table, with the raw data and a pencil and paper, Associate Professor Jette Thykær travelled with her family to work at PNNL for six months in 2008. Her knowledge on *A. niger*, along with CMB's noted competencies in fermentation technology, microbial physiology, metabolic mapping and other systems biology applications, complemented PNNL's expertise in proteomics and the acid-producing *A. niger*. In addition, PNNL has the luxury of generous governmental funding for its projects, but lacks hands to do the work, and Thykær welcomed the opportunity to spend a few months doing only bench work. Collectively, the goal of the collaboration project between CMB and PNNL, titled *"Aspergillus niger* proteomics – a systems biology approach for cell factory design", was to gain knowledge about the fundamental process of acid production, so that ultimately, optimal commercial strains can be designed. Financial support for the project came from the Danish Research Council.

A. niger produces acid as a defence, to inhibit the growth of nearby microbes, and give *A. niger* a competitive advantage. In addition to citric acid, the strain produces other compounds, like oxalic acid and gluconic acid, depending on the conditions. For both practical and basic research reasons, Thykær and her colleagues wanted to know what regulates the production of different acids under different conditions. Knowing the regulatory map that triggers production of a particular type of acid is the first step toward manipulating it.

To obtain some baseline information, Thykær and collaborators in Scott Baker's lab at PNNL initiated a study of the manganese switch *A. niger* growth and acid production. In the absence of manganese, the strain produces citric acid. When manganese is added, the strain changes morphology, becoming more filamentous, and stops producing citric acid. With CMB's expertise in fermentation providing the starting materials, the CMB and PNNL labs began an extensive transcriptomic and proteomic analysis of the two growth and acid-producing states. Working at PNNL gave Thykær access to their particularly effective proteome analysis system, which performs statistical analysis on small amounts of isolated proteins, identified by mass spectrometry, thereby bypassing the gel electrophoresis step of other proteomic methods. In the PNNL technique, all cell proteins are collected in an extract, cleaved

proteolytically and subjected directly to mass spec. The experimental design is more straightforward and less error-prone than other proteomic techniques, and the statistical analysis allows for quantitation.

The project is now in the data analysis phase. In a continuation of the manganese switch research, student Lars Poulsen carried out a Master's project focusing on identifying potential key regulators of acid production. As part of his thesis work, Lars also travelled to PNNL, where he was involved in constructing strains that will be used to test these regulatory candidates, and the trip allowed him to experience work and life in another lab and another culture. Lars just turned in his Master's thesis, and will continue to work on *A. niger* fermentation while applying for PhD opportunities.

In addition to the practical laboratory experience and the wealth of data generated in only a few months of collaboration, Thykær published two articles with the Baker lab, one identifying essential metabolic pathways in *A. fumigatus* using a metabolic flux model developed by CMB post-doctoral researcher Mikael Rørdam Andersen, and a commentary on the need to continuing fungal genome sequencing in the future. Thykær emphasizes the additional benefits she and CMB gained through the collaboration. "Doing the work in another lab allows you to look at your own system from a different perspective and see things you wouldn't see if you didn't go and experience it through work with a collaborator," she says. Thykær also welcomed the opportunity to introduce her family to a different culture, noting that her children loved dressing up and collecting candy on Halloween, and her family even enjoyed the over-the-top experience of watching the championship game of American football, at a Superbowl party. That's definitely an experience with collaborators that you can't get by phone or email.

Staff

Director
Morten Kielland-Brandt, Professor

Faculty
Andersen, Birgitte, Associate professor
Frisvad, Jens Christian, Professor
Hansen, Michael E. Assistant professor
Hobley, Tim, Associate professor
Karhumaa, Kaisa, Assistant professor
Lantz, Anna Eliasson, Associate professor
Larsen, Thomas Ostenfeld, Associate professor
Mijakovic, Ivan, Associate professor
Mortensen, Uffe, Associate professor
Nielsen, Kristian Fog, Associate professor
Nielsen, Michael L. Assistant professor
Panagiotou, Gianni, Assistant professor
Patil, Kiran, Assistant professor
Thrane, Ulf, Professor
Thykær, Jette, Assistant professor
Søndergaard, Ib, Associate professor

Post docs
Andersen, Mikael R.
Borodina, Irina
Bapat, Prashant M.
Hansen, Bjarne G.
Mapelli, Valeria
Nielsen, Jakob B.
Maury, Trine L.
Maury, Jerome
Pedersen, Mona H.
Phipps, Richard K.
Quriós, Manuel
Siewers, Verena
Vemuri, Goutham

PhD students
Albertsen, Line
Asadollahi, Mohammad A.
Brochado, Ana Rita
Brogaard, Katrine
Carlsen, Simon
Chen, Xiao
Faustrup, Helene
Formenti, Luca
Hallwyl, Swee
Johansen, Maria
Kjeldsen, Kjeld R.
Kold, David
Krogh, Astrid M.
Lehmann, Linda
Mapari, Sameer
Meijer, Susan
Mogensen, Jesper M.
Mølgaard, Louise
Olivares, Roberto
Oliveira, Ana Paula
Otero, José Manuel
Ottow, Kim
Papini, Marta
Pedersen, Lasse
Petersen, Trine L.
Piddocke, Maya P.
Poulsen, Tine R.
Rank, Christian
Rasmussen, Rie R.
Rueksomtawin, Kanchana
Rønnest, Mads H.
Salazar Pena, Margarita
Sohoni, Sujata
Storm, Ida D.
Sørensen, Jens L.
Sørensen, Marie
Tavares, Sabina
Usaite, Renata
Vongsangnak, Wanwipa
Wattanachaisaereekul, Songsak
Zhang, Jie
Ödman, Peter

Research assistants
Chumnanpuen, Pramote
Hansen, Vesna
Jose, Dinto
Mogensen, Jesper
Papadakis, Emmanouil
Partow, Siavash
Rattleff, Stig

Technical and administrative staff
Abdellatif, Mohammad, Technician
Andersen, Dorthe, Secretary
Andersen, Taja, Lab. assistant
Asueva, Anja A., Lab. assistant
Bro, Trine, Head of administration
Christiansen, Lene, Technician
Jakobsen, Hanne, Lab. manager
Jakobsen, Simo, Lab. trainee
Jensen, Anni, Technician
Johansen, Tina, Lab. manager
Kampp, Thomas, IT and Database manager
Karsbøl, Birgitte, Secretary
Knoth-Nielsen, Lisette, Technician
Kornholt, Martin E., Lab. trainee
Krøger, Elisabeth, Lab. manager
Laursen, Jytte V., Communications officer
Lyhne, Ellen K., Technician
Mortensen, Jette, Technician
Mylord, Martin, Lab. assistant
Svendsen, Lou, Office assistant
Wass, Anne, Office assistant
Winther, Pernille, Secretary

Guests
Abreu, Lucas, PhD student
Basilio, Carmo, PhD student
Copetti, Marina V., PhD student
James, Jemila, PhD student
Lages, Nuno, PhD student
Montagud, Arnau, PhD student
Polizzotto, Rachele, PhD student
Rebacz, Blanka, PhD student
Wasielewska, Joanna, PhD student