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***Review:***

# Global nutrient profiling by Phenotype MicroArrays: a tool complementing genomic and proteomic studies in conidial fungi*

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**Abstract:** Conidial fungi or molds and mildews are widely used in modern biotechnology as producers of antibiotics and other secondary metabolites, industrially important enzymes, chemicals and food. They are also important pathogens of animals including humans and agricultural crops. These various applications and extremely versatile natural phenotypes have led to the constantly growing list of complete genomes which are now available. Functional genomics and proteomics widely exploit the genomic information to study the cell-wide impact of altered genes on the phenotype of an organism and its function. This allows for global analysis of the information flow from DNA to RNA to protein, but it is usually not sufficient for the description of the global phenotype of an organism. More recently, Phenotype MicroArray (PM) technology has been introduced as a tool to characterize the metabolism of a (wild) fungal strain or a mutant. In this article, we review the background of PM applications for fungi and the methodic requirements to obtain reliable results. We also report examples of the versatility of this tool.

**Key words:** Biolog Phenotype MicroArray, Mitosporic fungi, Carbon metabolism, *Trichoderma*, *Aspergillus*, Biotechnology
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## 1 Introduction

Conidial fungi (mitosporic Dikarya, also informally called imperfect fungi or Fungi Imperfecti), commonly known as molds and mildews, represent a diverse and economically important biological group of organisms that include animal and plant pathogens, as well as organisms used in biotechnology. Some conidial fungi are responsible for the production of industrially important enzymes, antibiotics, secondary metabolites and foods (e.g., cheeses, sake, and soya sauce), whereas others produce toxins contaminating food products and/or resulting in food spoilage. Conidial fungi are capable of metabolizing a wide variety of nutrients. This characteristic has been exploited extensively to study metabolic pathways and their regulation in model fungi such as *Aspergillus nidulans* and *Neurospora crassa*. Their importance for humankind has consequently led to an increasing number of fungi for which genomic sequences are now available (>130 at http://www.ncbi.nlm.nih.gov/genomeprj as noticed on Dec. 15, 2009). Genome sequencing, however, is only the first step towards the identification and validation of the function of the genes of an organism. To understand the information stored in the genome, one must understand the production of knockout mutants and the consequence for the organism biology, learn the conditions for the expression of the gene, and have knowledge of the stability, compartmentation, and modification of the gene's translation product. To this end, "high-throughput methods", capable of studying all these traits, have and are being developed. For many conidial fungi, facilitated methods for production of knockout mutants have already been established

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(Sweigard *et al*., 1999; Chaveroche *et al*., 2000; Hamer *et al*., 2001), but new approaches for wide-domain functional characterization of the gene of interest need further elaboration.

In addition to investigation systems, which focus on specific genes, pathways, or processes, functional genomic methods attempt to study the impact of altered genes on the phenotype of the organism, using technologies that provide a cell-wide perspective. The most well known "global" approaches are DNA-microarrays for transcriptomics (Fodor *et al*., 1993; Kahmann and Basse, 2001) and two-dimensional gel electrophoresis (O′Farrell, 1975) for proteomics. These technologies allow for global analysis of the information flow from DNA to RNA to protein. To comprehend, however, how the gene initially is encoded in the genome is ultimately displayed at the cellular level; the phenotype must be considered. Phenotype is the manifested attribute of an organism, the joint product of its genes and their environment during ontogeny. As such, phenotypes are the ultimate goal in strain improvement in biotechnology during the screening of fungi for new processes or products. Therefore, a (semi)high-throughput and comprehensive phenotypic assay would greatly accelerate a functional genomic approach.

Since macronutrients are the major determinants of the fungal phenotype, a comprehensive profiling system should ideally include the maximum number of various nutrient sources in a single assay. This would contain some hundred carbon sources, and fifty to hundred nitrogen sources, sulphur and phosphorous sources, and eventually also include different pH and aeration conditions. Such a maximized system could be prepared in 5–7 microtiter plates with each well containing a standard medium specific for one particular phenotype (e.g., utilization of a sucrose), and allows the quantitative analysis of growth and/or metabolism. Unfortunately, custom synthesis of this type of comprehensive phenotype analysis system is laborious, and probably only feasible with the aid of laboratory robotics. The Phenotype MicroArray™ (PM) system (Bochner *et al*., 2001; Bochner, 1988; 2003) (Biolog Inc., Hayward, CA), however, offers a reasonable compromise and has frequently been used for the analysis of bacteria and yeast mutant strains (http://www.biolog.com/mID_section_13.html for bibliography). Kubicek *et al*. (2003) applied carbon source PMs to soil fungi, thereby detecting species-diagnostic characters in a collection of *Trichoderma* (teleomorph *Hypocrea*) isolates from Southeast Asia. Tanzer *et al*. (2003) demonstrated how global nutrient utilization analysis can be used to elucidate the effects of either genetic alterations or chemical treatments on *Aspergillus nidulans*, *Aspergillus fumigatus*, *Magnaporthe grisea*, and *Mycosphaerella graminicola*. In addition, Druzhinina *et al*. (2006) have applied PMs to investigate phenotypes of various wild-type and mutant strains of *Hypocrea jecorina* (anamorph *Trichoderma reesei*). The results presented in these three studies demonstrate that PMs indeed offer an ideal complement for the phenotypic characterization of gene knockout strains towards understanding genomic and proteomic data.

Here we review the applications of PMs to conidial fungi, and discuss the utility of this system for the high-throughput analysis of global phenotypes and utilization of particular nutrients.

## 2 Principle and reliability of Phenotype MicroArrays

During the initiative on functional genomics of *Saccharomyces cerevisiae* as a model system, two groups pioneered the testing of a large number of strains against 96 (Ross-Macdonald *et al*., 1999) or 288 (Rieger *et al*., 1997; 1999) phenotypes. In these studies, growth of yeast strains was tested on agar surface. It was problematic because scoring of growth on agar is prone to subjectivity and difficult to scale for high-throughput analysis. Several other researchers have tested their strains by cultivating them on a hundred or more different nutrient media compositions. Bochner (1989) first demonstrated a technique for global analysis of cellular phenotypes using microplates. He advocated the testing of phenotypes using cell respiration as a reporter system. The principle of this assay is the use of a tetrazolium dye (tetrazolium violet), which is reduced by the action of succinate dehydrogenase, enabling the quantification of respiration. Reduction of this dye results in formation of a purple color with a maximum absorbance at 490 nm. The reaction is essentially irreversible; therefore, the resulting dye accumulates in the well over a period of incubation, amplifying the signal and

integrating the amount of respiration over time. Although this assay works well in prokaryotes and yeasts (Bochner *et al*., 2001; Singh, 2009), color formation in imperfect fungi does not always coincide with growth (Fig. 1). Consequently, both Tanzer *et al*. (2003) and Druzhinina *et al*. (2006) quantified growth by mycelial production reading the optical density at 750 nm ($OD_{750}$).

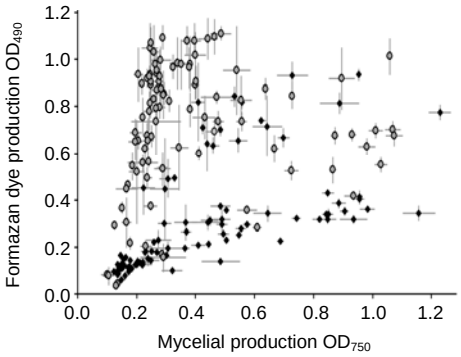


**Fig. 1 Correlation between formazan dye production and mycelial density**

Formazan dye production was estimated as difference between optical density (OD) values at 490 and 750 nm. Mycelial density directly corresponds to the OD value at 750 nm. Grey circles show the result of the experiment with *Hypocrea jecorina* QM 6a, and black diamonds indicate values from an experiment with *Hypocrea atroviridis* P1 strain. Vertical and horizontal error bars illustrate standard deviation calculated for three independent experiments per each strain

To achieve reproducible $OD_{750}$ measurements for certain fungal species, both the media for inoculum production and inoculum concentration had to be optimized. As an example, for *A. nidulans* and *M. graminicola*, the inoculum densities that resulted in the least variation ranged from $1.25\times10^5$ to $5\times10^5$ spores/ml, while for *M. grisea* the optimal inoculum concentration was strictly at $4\times10^5$ spores/ml (Tanzer *et al*., 2003).

Furthermore, contrary to endpoint assays absorbance data need to be collected over the incubation period to generate complete growth curves for each nutrient source. This is necessary because, for example, different carbon sources result in different growth kinetics (Fig. 2), and assessing growth only at a single time point would eventually be indicative of the early growth phase in one case and the phase of already terminated growth in another.

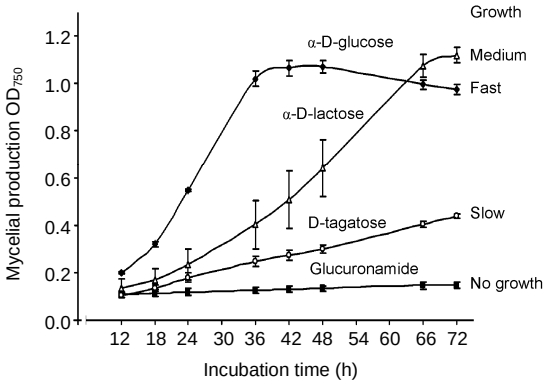


**Fig. 2 Four types of growth patterns revealed for *Hypocrea jecorina* (strain QM 6a) observed on Biolog FF MicroPlate**

D-glucose (black diamonds) exemplifies the group of quickly utilised carbon sources, which resulted in maximal biomass values already after 40 h of incubation. D-lactose (open triangles) is typical for carbon sources with medium utilisation rates, which gave maximal mycelium production after 3 d of growth. D-tagatose (open circles) belongs to the group of carbon sources, which provided only slow growth, and still commenced for longer than 72 h. Glucuronamide (black squares) is a compound, which does not support growth of *H. jecorina* QM 6a. Error bars correspond to standard deviation values calculated after three independent experiments

In addition, careful visual monitoring of cultures in test wells over the incubation period is essential: several carbon sources were found to initiate the early sporulation (e.g., 48 h of growth on γ-aminobutyrate for *H. jecorina*), which was reflected by increase in absorbance readings (Druzhinina *et al*., 2006). Careful examination of the time-dependent growth patterns on every nutrient composition as recommended above helps to eliminate this problem.

Finally, a last caveat would be the hyphal morphology. There are a number of carbon sources, which lead to aberrant morphology, and one would expect that thicker or irregularly branched hyphae would yield a different ratio of $OD_{750}$ vs. true biomass. Fortunately, this problem appears to be less important than theoretically predicted: we have tested the growth of *H. jecorina* wild-type strain in submerged culture, on agar surface and in PMs for several carbon sources with a potential morphological effect (e.g., L-sorbose), and generally found significant correlation between them. Therefore, $OD_{750}$ values are equivalent means to measure growth of conidial fungi on PM. Considering all the preconditions discussed

above, the reproducibility of PMs is very high. We have tested 17 wild-type and mutant strains of *H. jecorina* in three independent experiments when turbidity (mycelial production) was measured at several time points (Druzhinina *et al*., 2006). The subsequent statistical analysis did not detect any significant difference between corresponding plates in both cases when values for all carbon sources were averaged or tested individually, although the variable of time was always significant and has revealed three clusters attributed to spore germination, linear hyphal growth, and phase of sporulation and/or growth saturation. In spite of the high reproducibility of PMs, three or more independent tests are required as a precautionary measure against possible air-born contamination of test well(s), which might take place during readings at early growth stages or when the slow growing fungi are investigated.

### 2.1 Intra- and inter-specific variability in carbon source utilization in conidial fungi

Well studied filamentous fungi genera, e.g., *Aspergillus*, *Neurospora*, *Hypocrea* and *Acremonium*. are known to have a wide range of substrate assimilation (Caddick *et al*., 1994; Tanzer *et al*., 2003; Druzhinina *et al*., 2006; Hoyos-Carvajal *et al*., 2009). For species of genus *Hypocrea*/*Trichoderma*, rapid growth as well as ability to assimilate diverse substrates is a great advantage that allows this genus to colonize many ecological niches in the world. Kubicek *et al*. (2003) compared the inter-specific variability of carbon source utilisation profiles in *Hypocrea*/*Trichoderma*, and confirmed that isolates of most species formed defined clusters, thus proving the applicability of this method in species identification. This approach was then integrated in a description of *T. brevicompactum* sp. nov. by Kraus *et al*. (2004).

PMs were also applied to study the differentiation of two morphologically and ecologically very similar marine species *Dendryphiella arenaria* and *D. salina* (dela Cruz *et al*., 2006), when the qualitative difference in utilization of four carbon sources confirmed the species divergence inferred from genetic data. Moreover, PMs could also differentiate subpopulations within *Dendryphiella* species in relation to their geographic origin (dela Cruz *et al*., 2006).

Nutrient source profiling was also applied by Komon-Zelazowska *et al*. (2007) to determine the evolutionary context of two genetically closely related, but phenotypically very different species (*Trichoderma pleuroticola* and *T. pleurotum*) that cause green mold disease in oyster mushroom (*Pleurotus ostreatus*) farms worldwide. This assay revealed generally impaired growth of *T. pleurotum* on numerous carbon sources according to enhanced assimilation of those by *T. pleuroticola*, which thereby showed very similar metabolic characteristics to its phylogenetically close members in the Harzianum clade of *Hypocrea*/*Trichoderma* (*T. harzianum* and *T. aggressivum*). It was shown that the highest assimilation rates for *T. pleuroticola* occurred on *N*-acetyl-D-glucosamine and quinic acid, which could be useful for differentiation of two causative agents of *Pleurotus* green mold disease. *T. pleuroticola*, on the other hand, can be distinguished from *T. aggressivum* (green mold disease on *Agaricus*) by the inability of the latter to utilize α-ketoglutaric, L-malic, and succinamic acids (Komon-Zelazowska *et al*., 2007). Thus, these kinds of assays are important sources of complementary data for precise species identification and as a starting point for uncovering the ecological niche of a fungus.

Furthermore, as shown in Fig. 3 (biochemical and physiological groups of carbon sources are given in Fig. 1A), PM analyses of different species displayed significant intraspecies diversities (*H. jecorina*) or were conserved (*H. atroviridis*) (Seidl *et al*., 2006; Friedl *et al*., 2008b). It was interesting to see that for *H. jecorina* the level of this variability was in the same range as that obtained by DNA-mediated transformation (Druzhinina *et al*., 2006). It may suggest that the difference between *H. jecorina* isolates is due to the sexual recombination, which is possibly less frequent in *H. atroviridis*.

Yet not every study could successfully confirm species differentiation: we tested the hypothesis that opportunistic strains of *T. longibrachiatum* isolated from the lungs of immunocompromised patients may represent specialized potentially clonal subpopulations within this species, which would allow us to identify specific genetic markers for their diagnosis. In contrast, the results from this work provide clear evidence that not only one, but two, genetically different species *T. longibrachiatum* and *H. orientalis* cause infections, and that clinical and environmental isolates reveal surprisingly consistent PMs (Druzhinina *et al*., 2008).

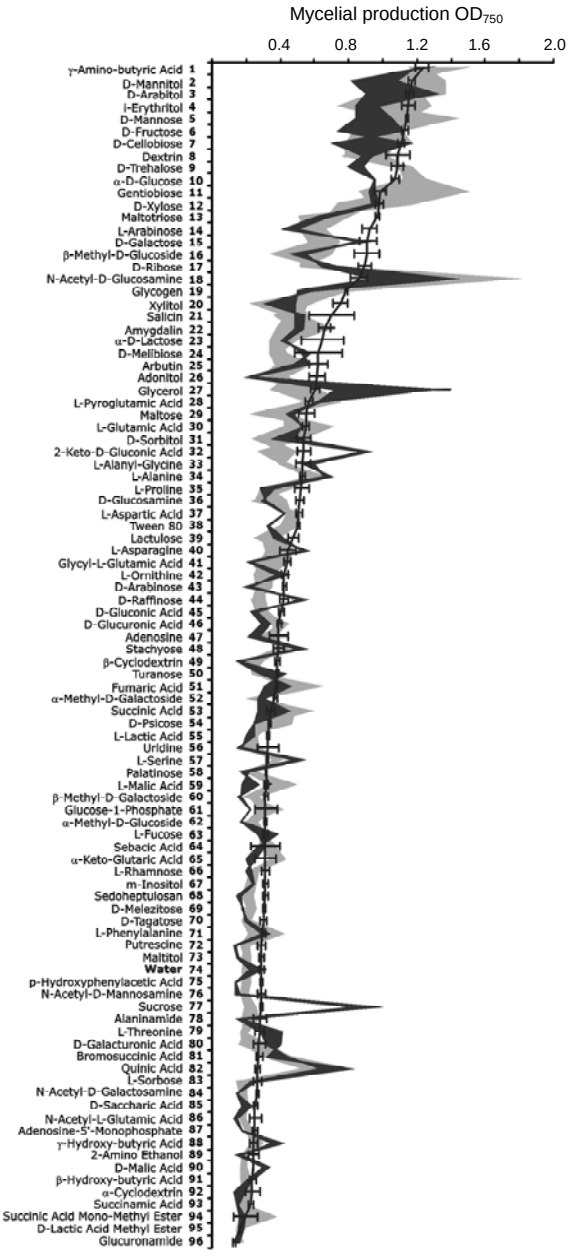


**Fig. 3 Metabolic profiles of *H. jecorina* and *H. atroviridis* as inferred by Biolog Phenotype MicroArray analysis after 48 h of incubation**

Light grey shadow corresponds to the summed metabolic profile of five wild-type strains of *H. jecorina*. Dark grey shadow indicates the combined profile of three *H. atroviridis* strains. The solid line corresponds to the reference strain *H. jecorina* QM 6a, which has a unique physiological profile. Error bars indicate standard deviation values estimated based on tree independent experiments

In the manner of species identification, Hoyos-Carvajal *et al*. (2009) recently examined the diversity of *Hypocrea/Trichoderma* in neotropic regions using morphological, genetic, as well as metabolic approaches, proving that the latter should not to be omitted in the recognition and characterization of new species or in the analyses of phylogenetic relationships within a fungal genus. Hoyos-Carvajal *et al*. (2009) reported that in *T. asperellum*, one of the most abundant species found in neotropical regions, two distinct genotypes were supported by carbon source utilization profiles: strains of the *T. asperellum* clade A originating from forests grew on very poor substrates, whereas strains from clade B in situ associated with crops preferred rather readily available substrates such as sugars. The predominance of *T. harzianum* can be attributed to a relatively wide variety of utilized carbon substrates, as reported by Hoyos-Carvajal *et al*. (2009), yet three new species morphologically similar to *T. harzianum* showed atypical metabolic profiles in the cluster analysis as well as a separate lineage in the sequence analysis of rRNA markers.

Moreover, a screening test for rapid differentiation of strains of the superior chitinase producing fungi attributed to *T. harzianum* sensu stricto clade with Biolog Phenotype MicroArrays was developed by Nagy *et al*. (2007), revealing that these fungi are unable to grow on *N*-acetyl-D-mannosamine. This fact was used to establish a simple test for directed screening for chitinase-producing strains (Nagy *et al*., 2007).

PM analysis aids, however, in the detection of intra- and inter-specific variability of conidial fungi, thereby offering a means for characterizing species, individual strains, and ecological groups.

## 2.2 Identification of phenetic differences in non-transformed mutant strains

Many of the classical fungal mutant strains used in biotechnology were selected after chemical or physical mutagenesis solely based on improved product formation, while the biochemical or genetic nature of the mutation has rarely been revealed. An example is *T. reesei* QM 6a (teleomorph *Hypocrea jecorina*), which was subjected to a classical mutagenesis by a series of exposures to radiation with a linear accelerator. The resulting mutants displayed a two- to four-fold increase in cellulolytic activity. We have tested whether PMs would be capable of identifying the possible changes, which had accompanied

this mutagenesis (Druzhinina *et al*., 2006). The data showed that, in general, mutagenesis left the carbon source utilization profile of the respective strains remarkably unchanged, thus indicating that an improvement of cellulase formation was due to mutation of very specific intracellular targets, such as those involved in inducer formation or reception. Some subtle differences, however, could be observed: increased cellulase formation was shown to correlate with a decreased growth on adonitol (=D-ribitol), 2-ketogluconate and γ-aminobutyric acid and increased growth rates on D-sorbitol and saccharic acid. The metabolism of two of these compounds (2-ketogluconate, saccharic acid) in *H. jecorina* is unknown, thereby preventing an interpretation of the observed effect. The two others (γ-aminobutyric acid, D-sorbitol), however, may indeed offer interesting insights: γ-aminobutyric acid formation is up-regulated during the early phase of conidiation in *Trichoderma*, and exogenous addition of γ-aminobutyrate stimulates sporulation in *T. reesei* (unpublished data). Since cellulase formation is triggered by sporulation (Kubicek, 1987), γ-aminobutyrate may constitute a link between cellulase induction and conidiation. On the other hand, the increased utilization of D-sorbitol by the improved producer strain QM 9414 may be linked to enhanced formation of L-sorbose, an inducer of cellulase formation in *H. jecorina* (Nogawa *et al*., 2001), because it is formed from D-sorbitol by the respective nicotinamide adenine dinucleotide phosphate (NADP)-dependent ketose reductase (Seiboth *et al*., 2007). While the importance of these two findings for cellulase production must be verified by reverse genetics, they have nevertheless pointed to two biochemical reactions, which may have a major impact on cellulase formation, and would have remained undetected without PM analysis.

### 2.3 Biomining for new biocatalysts and improved producer strains

Most of the fungal strains used today in the production of enzymes, secondary metabolites, and other organic chemicals have been screened by testing for either formation of the product itself or for genetic alterations assumed to cause improved production such as carbon catabolite derepression, e.g., by using resistance to 2-desoxiglucose for cellulase overproduction in *H. jecorina* (Montenecourt and Eveleigh, 1979). Improved production, however, may also be due to changes of unknown or unexpected physiological properties, detection of which may therefore offer new methods for the direct screening of improved producer strains. To this end, we have recently used carbon source PMs to correlate the ability to secrete high chitinase activities with the carbon source assimilation profile in *Trichoderma*. In line with the comments made above, we in fact observed a statistically relevant correlation between the assimilation rate of lactic acid methyl ester and chitinase activity (unpublished data). While the physiological meaning of this correlation is not yet understood, lactic acid methyl ester is a valuable indicator of the chitinolytic potential of *Trichoderma* species.

While PM primarily provides information about the growth rate on different nutrients, the integration of these data into a biochemical map could also lead to the detection of pathways or enzymatic steps, which are unique for a particular strain, species or genus. One barrier towards achieving this is that not all of the biochemical pathways occurring in filamentous fungi are as yet understood to the level that the implementation of this type of network would be feasible. Some biotechnologically useful information can, however, be extracted already at this stage. For example, *N*-acetyl-mannosamine is a very poor carbon source for some species and strains of *Hypocrea*/*Trichoderma* such as *H. jecorina*, although they all assimilate *N*-acetyl-glucosamine very well. The majority of strains of *H. lixii*/*T. harzianum* also utilize *N*-acetyl-mannosamine. This is due to the formation of the enzyme *N*-acetylglucosamine epimerase, which converts *N*-acetyl-glucosamine into *N*-acetyl-mannosamine as shown in Fig. 4 (Kowal and Wang, 2002). Since the reaction of this enzyme is essentially in equilibrium, it can, vice versa, be added to the in vitro degradation of chitin by *Trichoderma* chitinase, which would then ultimately lead to the accumulation of *N*-acetyl-mannosamine, a high-value product needed for the biocatalytic synthesis of sialic acid-like compounds used in various therapies (Blayer *et al*., 1999; Schauer, 2000; Keppler *et al*., 2001). Yet, using the PM method for detecting the differentiation between chitinase-overproducing strains among *H. lixii*/*T. harzianum* (Nagy *et al*.,

2007), the lack of *N*-acetyl-β-D-mannosamine utilization was present in the superior chitinase producer strains.

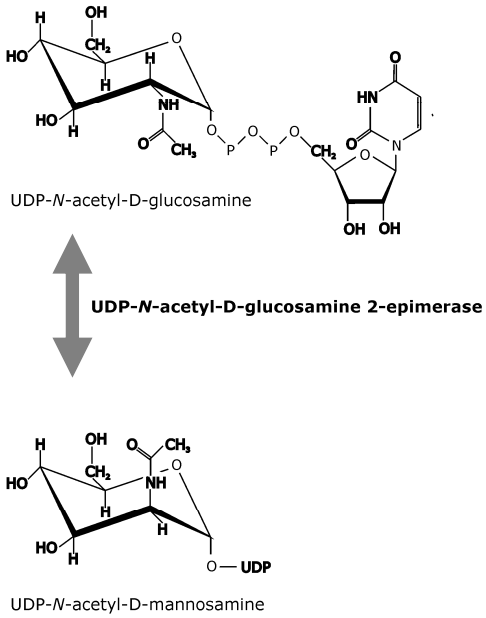


**Fig. 4 Conversion of *N*-acetyl-glucosamine into *N*-acetyl-mannosamine by enzyme *N*-acetyl-glucosamine epimerase [modified from Kowal and Wang (2002)]**

Furthermore, a screening system consisting of a combination of Biolog PM and specific enzyme activity measurements using a chromogenic substrate (Seidl *et al*., 2006) was developed to identify carbon sources that trigger β-*N*-acetyl-D-glucosaminidase (NAGase) formation in *Hypocrea atroviridis*. Those data were compared with transcript patterns of *nag1* and *nag2*, two genes encoding the NAGase Nag1 and Nag2, in the wild-type and an *H. atroviridis* Δ*nag1* strain (Seidl *et al*., 2006). No differences in the phenotype of these two strains were observed. As the result of the screening Δ*nag1* strain showed a strong reduction of NAGase activity on most carbon sources compared to the wild-type. A number of carbon sources that clearly enhanced NAGase activity were detected, mostly α-glucans, like glycogen, dextrin and maltotriose, and several oligosaccharides. The authors claimed that, since this kind of glycosidic linkage is present in the cell walls of the majority of ascomycetes, these carbon sources can be a part of a mechanism by which *H. atroviridis* senses the presence of a host cell wall containing chitin. Moreover, the comparison of NAGase formation between wild-type and mutant *H. atroviridis* strains showed that the reduction of this enzyme activity varied greatly among different carbon sources, and that the ratio was growth-phase dependant (Seidl *et al*., 2006).

Overall, the screening system of the combination of PMs and an enzymatic assay using a chromogene substrate established by Seidl *et al*. (2006) can be adapted for enzyme activity measurements of a variety of extracellular and cell wall-bound enzymes. Apparently, the differences in regulation between *nag1* and *nag2* would stay undetected without this tool. Seidl *et al*. (2006) propose this method as useful to monitor the expression of specific genes, even for encoding intracellular enzymes, by using appropriate promoter-fusion reporter systems.

### 2.4 Testing gene knockouts with expected phenotypes

DNA-mediated transformation is now a routine method applied to many fungi, and creation of knockout mutants by homologous integration, therefore, is a standard procedure. In order to obtain reliable knockouts, Southern analyses must be performed to confirm the homologous integration, and a regain of the phenotype of the parent strain by retransformation with the wild-type gene is also essential. Assessment of the wild-type and mutant phenotypes, however, is often laborious. PMs can provide a valuable tool here, as shown with an example of *H. jecorina*: we have previously investigated xylitol dehydrogenase gene (*xdh1*) for its possible involvement in D-xylose catabolism (Seiboth *et al*., 2003). Analysis of an *xdh1*-deletion mutant by PMs (Table 1) reveals confirmations that this mutant is partially defective in growth on L-arabinose and D-xylose, because its activity can be taken over by L-arabinitol dehydrogenase. PMs further show, however, that the mutant has impaired growth on D-sorbitol, whereas it has higher growths on erythritol and glycerol. The latter finding is not understood, but suggests an interplay of xylitol dehydrogenase with catabolism of shorter chain polyols, which needs to be taken into account when the physiology of these mutants is investigated.

Two mutant cellulase-hyperproducing strains NG14 and its direct descendant RUT C30, initially originating from *T. reesei* QM 6a, were newly tested

**Table 1 Utilization of selected carbon sources by *H. jecorina* QM 9414 and Δ*xdh1* transformant strains**

| Carbon source | 48 h | | | 66 h | | |
|---|---|---|---|---|---|---|
| | QM 9414 | Δ*xdh*1 | *P* value | QM 9414 | Δ*xdh*1 | *P* value |
| L-arabinose | 0.61 | 0.35 | <0.01 | 1.14 | 0.46 | <0.01 |
| D-cellobiose | 0.87 | 0.91 | 0.02 | | Not significant | |
| i-erythritol | 0.86 | 1.07 | <0.01 | 1.08 | 1.16 | 0.04 |
| Glycerol | 0.44 | 0.66 | 0.03 | 0.74 | 1.04 | <0.01 |
| D-sorbitol | 0.54 | 0.26 | <0.01 | 0.88 | 0.31 | <0.01 |
| Xylitol | 0.49 | 0.56 | <0.01 | | Not significant | |
| D-xylose | 0.94 | 0.47 | <0.01 | 0.94 | 0.75 | <0.01 |

to identify mutations in the genomes that led to cellulase production (le Crom *et al*., 2009). These two strains were identified on the basis of their superior cellulase activity as well as resistance to 2-deoxyglucose in the presence of glycerol (Seidl *et al*., 2008). Biolog PMs were used to compare the mutants with their wild-type strain QM 6a. It was shown that utilization of carbon sources glucose, D-xylose, D-arabinitol, mannitol, and the β-linked disaccharides gentiobiose and cellobiose is correlated with increased cellulose production (le Crom *et al*., 2009). The latter two carbon sources might reflect enhanced β-glucosidase activity, yet the rest of utilized carbon sources are catabolite repressing compounds and at this point, le Crom *et al*. (2009) stressed that it is not clear whether this is a consequence of the *cre1* mutation or it is due to the effect of other affected genes. Previously reported inverse carbon source utilization in correlation with enhanced cellulase production was detected by utilization of α-linked oligosaccharides and glycans (Seidl *et al*., 2008), and was complemented by the correlation between cellulase production and reduced growth on amino acids. le Crom *et al*. (2009) discussed this phenotype to be related to an ability of the superior cellulase production strains to use a higher portion of their amino acid pool for synthesis of secreted proteins versus growth.

## 2.5 Analysis of wide-domain regulatory processes

PMs are particularly well suited for the investigation of wide-domain regulatory processes, e.g., carbon catabolite regulation, due to the possibility of measuring growth on 96 different conditions simultaneously (carbon sources, nitrogen sources, stress inducers, pH control, and others). These factors have been intensively studied in *A. nidulans*, *N. crassa*, *H. jecorina*, and *Acremonium chrysogenum* (Bailey and Arst, 1975; Kudla *et al*., 1990; Dowzer and Kelly, 1991; Caddick *et al*., 1994; Strauss *et al*., 1995; Ilmén *et al*., 1996; Platt *et al*., 1996; Ravagnani *et al*., 1997; Marzluf, 1997; Wilson and Arst, 1998; Jekosch and Kück, 2000; Felenbok *et al*., 2001). In this type of experiment, growth of a parent strain and a respective mutant would be compared on every medium composition, which would reveal those particular nutrients whose utilization depends on the mutated wide-domain control gene.

### 2.5.1 Nitrogen repression

Tanzer *et al*. (2003) used this approach to investigate the effect of the *areA*-dependent nitrogen regulation on the utilization of various nitrogen sources by *A. nidulans*. The *areA* gene encodes a GATA family transcriptional activator mediating nitrogen metabolite repression, thus ensuring the utilization of ammonium and L-glutamine in preference to a variety of alternative nitrogen sources. Mutations in *areA* exhibit an extraordinarily diverse range of phenotypes when monitored for their effects on utilization of nitrogenous compounds. One of these alleles, *areA-102*, displays altered specificity of target promoter activation, which ultimately leads to loss-of-function, gain-of-function, or a wild-type phenotype based on its interaction with the target structural gene promoter (Kudla *et al*., 1990; Ravagnani *et al*., 1997). In comparison to wild-type strains on agar plates, strains carrying *areA*-102 show increased growth when urea, L-histidine, L-citrulline, L-aspartate, or L-glutamate is used as the sole nitrogen source and decreased growth when xanthine or uric acid is used as the sole nitrogen source (Arst and

Cove, 1973; Arst and Scazzocchio, 1975; Hynes, 1975; Kudla *et al*., 1990). Results obtained with PMs were largely in agreement with these observations. In contrast to them, however, growth on L-glutamate or L-aspartate in the *areA*-102 mutant was not higher than that in the wild-type, and growth on xanthine was not detectable. On the other hand, assimilation of quite a number of nitrogen sources was not altered, indicating that they are not a subject to the AreA-mediated nitrogen regulatory system. An interesting finding from this study was that several D-amino acids (D-alanine, D-valine, and D-lysine) were utilized more efficiently by the *areA*-102 mutant strain than by the wild-type strain, suggesting that this *areA* mutation elevates D-amino acid oxidase levels. Consistent data were obtained with another allele (*areA*-30), which is a revertant allele that also shows altered specificity of target promoter activation.

Tanzer *et al*. (2003) further used PMs to perform a large scale analysis of 21 *A. nidulans* strains containing various *areA* mutant alleles. The data from all test wells for each strain were used for cluster analysis based on overall nitrogen source utilization patterns. The data provided excellent insight into the severeness of the individual mutations, e.g., mutant *areA*-1903 or *areA*-2173, which contain the conservative G698A substitution (Wilson and Arst, 1998), clustered with the wild-type strain, because these mutants produce no apparent effect in growth tests on solid medium. Other mutations, particularly those leading to a truncated AreA protein, showed much more serious differences. This work therefore beautifully illustrates the use of PM to screen for essential and dispensable mutations in a regulatory protein.

### 2.5.2 Carbon catabolite repression

Carbon catabolite repression is another wide-domain regulatory circuit amenable to investigation by PM. Its most observed phenotype is due to the *creA*/*cre1* gene, which encodes a $C_2H_2$-zinc finger-containing transcriptional repressor (Dowzer and Kelly, 1991; Strauss *et al*., 1995; Ilmén *et al*., 1996). It ensures the utilization of glucose and some other carbon sources in preference to a large variety of other carbon compounds, which either yield less energy during assimilation (e.g., $C_2$-components), or require a considerable amount of energy for becoming assimilable (e.g., extracellular macromolecules, for which hydrolases must be exported via a secretory pathway).

To determine the utilization of which carbon sources is actually affected by the carbon catabolite repression, Seidl *et al*. (2008) investigated the differences in the growth of a *cre1*-truncated mutant strain of *H. jecorina* (RUT C30) (Ilmén *et al*., 1996) and the respective parent strain NG 14. The results (Fig. 5) show that the carbon catabolite-derepressed mutant strain displays a higher growth rate on D-arabitol, D-mannitol, D-fructose, D-trehalose, D-mannose, D-xylose, D-ribose, *N*-acetyl-D-glucosamine, γ-aminobutyric acid, and glycerol, indicating that all of them are under partial carbon catabolite repression, and that they all repress their own utilization to some extent. As expected, growth on glucose was not different and therefore it can serve as a control for these investigations. Interestingly, the *cre1*

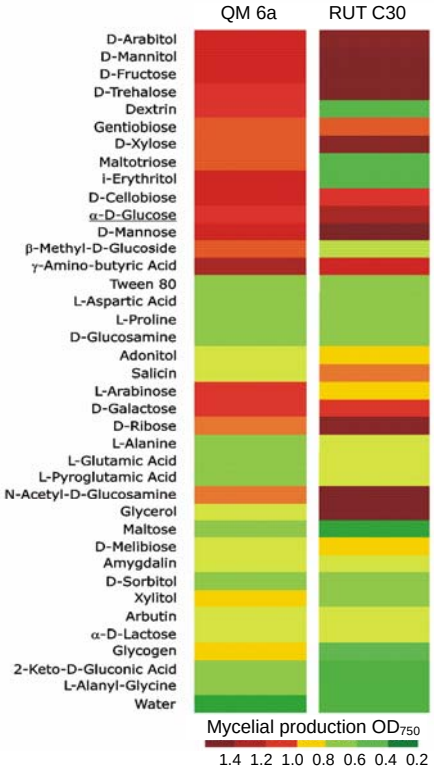


**Fig. 5 A phenetic map of carbon utilization patterns of *H. jecorina* QM 6a and the carbon catabolite-derepressed strain RUT C30 after the two-ways joining cluster analysis was applied to (i) carbon sources and (ii) fungal strains as two groups of variables. Only "fast" and "medium" carbon sources are shown. The respective growth ($OD_{750}$ after 48 h) is given by a corresponding color as indicated at the color scale**

mutation also results in significant changes in the other direction, e.g., the carbon catabolite-derepressed mutant had decreased the ability to grow on erythritol, dextrin, maltotriose, glycogen, and β-methyl glucoside. The biochemical nature of these observations is unclear, but indicates a dependence of carbon catabolite repression on assimilation of these carbon sources.

Seidl *et al*. (2008) also showed that the *H. jecorina* RUT C30 and its ancestor NG 14 lack an 85-kb genomic fragment, missing additional 29 genes comprising transcription factors, enzymes of the primary metabolism and transport proteins. They reported that these mutations are not linked to the *cre1* locus. Performing PM analysis, it was concluded that a greatly reduced growth of RUT C30 on L-arabinose, L-erythritol and also D-galactose indicates that one of the aldo-/keto-reductases identified as lacking in this strain could be involved in polyol assimilation (Seidl *et al*., 2008).

Tanzer *et al*. (2003) used an alternative, indirect approach to examine the effect of carbon catabolite repression on carbon source utilization in *A. nidulans*, *A. fumigatus*, *M. grisea*, and *M. graminicola*. The approach was based on the CreA-dependent repression of alcohol dehydrogenase, which renders these strains unable to grow on derepressing carbon sources in the presence of allyl alcohol (Bailey and Arst, 1975; Felenbok *et al*., 2001). Obviously, this method can be used only with fungi that are able to use ethanol as a sole carbon source, which is not the case for others, e.g., *H. jecorina* (C.P. Kubicek, personal communication). The validity of the approach for the species investigated, however, arises from the fact that in all four fungal species, the addition of allyl alcohol to the media containing D-glucose had a little effect on the growth levels whereas it was strongly inhibited in media containing the derepressing carbon source L-lactic acid. A closer examination showed that the derepressing class of carbon compounds could be further subdivided by comparing growth in the presence of different concentrations of allyl alcohol. *A. nidulans* and *A. fumigatus* were generally more sensitive to allyl alcohol toxicity than *M. grisea* and *M. graminicola*, the latter two species requiring, respectively, 25- and 2.5-fold higher concentrations of allyl alcohol to reduce growth on derepressing carbon sources (Tanzer *et al*., 2003). These data also revealed contrasting results in different fungi, e.g., while dextrin was derepressing in the *A. nidulans*, it was repressing in *A. fumigatus*, *M. grisea*, and *M. graminicola*. Additionally, several other carbon sources such as maltose, D-sorbitol, and D-trehalose were more strongly repressing in *A. fumigatus* as compared to *A. nidulans*, and a much larger number of carbon sources were strongly repressing in *M. grisea* and *M. graminicola*, e.g., D-trehalose, lactose, and D-raffinose. The fact that *Magnaporthe* and *Mycosphaerella* are less sensitive to allyl alcohol and are derepressed by fewer compounds suggests that the plant pathogens are poised to utilize a number of compounds as carbon sources rapidly whereas the aspergilli are initially more selective and at the same time can use a wider range of carbon sources. Tanzer *et al*. (2003) concluded that the regulation of nutrient utilization may evolve to favour adaptation to the certain environmental niches and thus may provide insights into the nutrients typically encountered by these fungi.

Even though the allyl alcohol resistance system cannot be used for fungi like *Hypocrea*/*Trichoderma*, it is still amenable to this type of global analysis by other means: 2-desoxyglucose (2-DOG) has been used as a nonmetabolizable agent conferring carbon catabolite repression in yeast and filamentous fungi, because it accumulates as 2-desoxyglucose-6-phosphate (Franzusoff and Cirillo, 1982; Randez-Gil *et al*., 1995). Therefore, 2-DOG has been used for the isolation of carbon catabolite derepressed mutants in *H. jecorina* and *Penicillium chrysogenum* (Montenecourt and Eveleigh, 1979; Farkaš *et al*., 1981; Barredo *et al*., 1988). A drawback of the use of 2-DOG, however, is that it also interferes with energy metabolism (e.g., it depletes the cells of adenosine-triphosphate (ATP) because of storage of phosphate in 2-DOG-phosphate and slowing down glycolysis). In order to take that into consideration, the influence of 2-DOG is examined at several different sublethal concentrations.

Brunner *et al*. (2008) analyzed G protein-coupled receptors (GPCRs) in a manner to learn more about the G protein signaling. With the help of in silico exploration of the genome database of the close relative *T. reesei*, four *H. atroviridis* GPCR-encoding genes were isolated and affiliated to the cyclic adenosine monophosphate (cAMP) receptor-like

family. Using Biolog PMs, it was shown that *gpr-1* silenced mutants exhibited reduced growth on several carbon sources, e.g., glucose and glycerol growth of the mutants was reduced by 70%. Furthermore, in comparison to the parental strain, the mutant was not able to grow on lactose and its isomerization product lactulose, and also showed scarce growth on maltose and raffinose. There were also some carbon sources that showed similar growth rate at both strains, indicating that the mutant's reduced growth is carbon source-dependent and generally ineffective. Overall, the characterization of the *H. atroviridis* Gpr-1 revealed that the GPCR influences conidiation, conidial germination and vegetative growth on many carbon sources (Brunner *et al*., 2008). The PM method aids to the exploration of the fundamental role of G protein signaling in *Trichoderma*.

### 2.6 Application of PMs in fungal photobiology

Contrary to the initial expectation, Friedl *et al*. (2008a) revealed that conidiation in *Hypocrea atroviridis* is primarily carbon source-dependent and that illumination plays a catalytic role in this process. They applied PMs to a wild-type strain of *H. atroviridis* as well as two mutant strains, with each the loss of function of the blue light regulators 1 and 2 (BLR-1 and BLR-2), respectively, responsible for stimulation of transcription activation. In the mutant strains Δ*blr-1* and △*blr-2*, conidiation in the darkness was significantly decreased on most carbon sources. Except for strong conidiation of Δ*blr-1* on D-sorbitol and Δ*blr-2* on glucuronic acid, the carbon sources that stimulated low conidiation in the mutant strains were mostly from the pool of those that contribute to strong conidiation in the parent strain (Friedl *et al*., 2008a). While observing this strongly impaired sporulation, it is notable that the mutations do not completely block sporulation but only greatly decrease it, and they still show (light insensitive) sporulation on some carbon sources (notably glucuronic acid and 2-ketogluconic acid). Friedl *et al*. (2008a) therefore hypothesized that the observed differences in levels of conidiation on different carbon sources are due to different redox potentials in the cell during growth. Thus, BLR proteins are not only involved in the photosensing, but also act as redox and oxygen sensors.

Friedl *et al*. (2008a) also concluded that the addition of cAMP induces the sporulation in darkness, as previously reported by Casas-Flores *et al*. (2004), but also showed that effect of cAMP is carbon source-dependent. The addition of cAMP induced conidiation in the parent and both *blr* mutant strains on some, but not all, carbon sources. Thus, the carbon source dependence of mutant strains was different from that of the parent strain. Only a small set of carbohydrates, polyols, and sugar acids allowed conidiation in darkness, whereas for most of them conidiation was strongly expressed in light. Moreover, under every condition tested (darkness, light, addition of cAMP, the loss of function of BLR-1/BLR-2) spore formation occurred only on carbohydrates and their derivates (polyols and sugar acids). Yet, the presence of light always enabled the sporulation only on those carbon sources which also empowered conidiation in darkness by increasing the speed or intensity of sporulation (Friedl *et al*., 2008a). There were a number of carbon sources (e.g., rhamnose, glucosamine and xylitol) detected where light did not further stimulate the spore formation.

Furthermore, Friedl *et al*. (2008b) showed that enhancement of mycelia growth on various carbon sources is highly light-dependent. Carbon source utilization by PMs showed that *Hypocrea atroviridis* growth is light-stimulated on 17 of 95 carbon sources, which are mainly metabolically related to cellulose and hemicelluloses and can be found in the upper soil litter layer (Friedl *et al*., 2008b). The light stimulation depends on the function of two blue light receptor proteins BLR-1 and BLR-2. Utilization of carbon sources in darkness was nearly the same in both knock-out mutants (Δ*blr-1* and Δ*blr-2*), and it was similar to that of the parent strain. But comparing carbon utilization profiles to those exposed to rhythmic and constant illumination, Friedl *et al*. (2008b) concluded that the mutant strains are not photostimulated, but in contrast are inhibited by light, especially in Δ*blr-2*. Evocation of oxidative stress response in darkness imitates the photostimulation on 9 of the mentioned 17 carbon sources, and this effect is fully dependent on the function of BLR-1. The resistance of both *blr* mutants to conditions of oxidative stress is therefore carbon source-dependent and is notably weaker compared to the wild-type strain. Friedl *et al*. (2008b) concluded that light in combination with the availability of litter-specific carbon sources serves as a signal for the fungus to be above

ground, thereby stimulating fast growth in order to produce a maximum of propagules in the shortest time. Furthermore, they deduced that this process involves oxidative stress response and the two blue light receptor proteins BLR-1 and BLR-2, the former playing the major role.

To determine the role of the light regulatory protein ENVOY, Schuster *et al*. (2007) used PMs as an aid to their global screening for genes, which are specifically affected by light in the fungus *H. jecorina*, an important producer of cellulases and hemicellulases. The expression of its cellulase genes is partially susceptible to carbon catabolite repression. They showed that ENVOY acts as a light-independent repressor for several genes and it is crucial for normal growth in light on several carbon sources, but is not able to fully execute its regulatory function when overexpressed in darkness. Higher growth rate of the *H. jecorina* QM 9414 than that of the mutant strain env1$^{PAS-}$ measured in the light indicated that the growth rate is dependent on ENVOY since no such stimulation was observed in the mutant strain (Schuster *et al*., 2007). Moreover, Schuster *et al*. (2007) found that, with the exception of growth on γ-aminobutyric acid, mutant strain growth rate is always lower in light than in the dark. They also reported that the growth rates of two strains compared separately in light and darkness differ significantly more in the light, indicating that light inhibits growth of strain env1$^{PAS-}$. On the other hand, it was shown that growth in darkness (with the exception of glycerol) was poorly affected. They claimed an *env1*-dependent enhancement of energy metabolism, and thus biomass formation by light as well as a negative effect of light on *H. jecorina* in the absence of functional ENVOY. The inhibitory effect of light in the absence of ENVOY is carbon source-dependent since the inhibition by light in the mutant strain was not observed on all carbon sources (Schuster *et al*., 2007).

### 2.7 Development and analysis of action of chemical inhibitors

Many fungi are pathogens of plants, animals or human beings. Therefore, screening for novel chemicals capable of inhibiting these species is an important issue (Vitale *et al*., 2005). This is also of particular significance to the increasing number of cases in which non-human-pathogenic fungi such as *Trichoderma* or *Fusarium* have caused fatalities in immunocompromised patients (Yeo and Wong, 2002; Sampathkumar and Paya, 2001). Many of these isolates proved to be resistant to the common clinical fungicides such as fluconazole and 5-fluorocytosine (5FC), amphotericin B, tetraconazole and ketoconazole (Ragnaud *et al*., 1984; Campos-Herrero *et al*., 1996; Tanis *et al*., 1995; Guarro *et al*., 1999; Hennequin *et al*., 2000; Antal *et al*., 2002; Avery, 2004). Thus, the development of new agents is essential. This development also must accompany the identification of action of the compound, for which PM offers an attractive possibility. To show the utility of PM for chemical inhibitor analysis, Tanzer *et al*. (2003) compared the responses of *A. nidulans* and *M. grisea* to the addition of the growth inhibitor glufosinate. Glufosinate (phosphinothricin) is a herbicidal compound that has also been shown to inhibit animal and fungal growth (Avalos *et al*., 1989; Pall, 1993; Kutlesa and Caveney, 2001). It is an analogue of L-glutamate that inhibits glutamine synthetase, thereby preventing the synthesis of glutamine and consequently the synthesis of numerous other nitrogenous compounds required for growth. Based on its mode-of-action, the addition of glutamine should abrogate the inhibitory effects of glufosinate by bypassing the need for glutamine synthetase. Tanzer *et al*. (2003) tested the effect of glufosinate on growth of *A. nidulans* and *M. grisea* in the presence of different nitrogenous compounds. *A. nidulans* showed near wild-type levels of growth on glutamine and glutamate, which successfully competes for the inhibitor, but no growth when ammonium, nitrate or nitrite was used as the nitrogen source. Thus, Tanzer *et al*. (2003) showed that L-aspartate was not an effective competitor and glutamine-containing dipeptides were apparently unable to supply sufficient glutamine to overcome the toxicity. *M. grisea* was able to grow normally in the presence of L-glutamine or L-glutamate, while growth was inhibited when ammonium, nitrate or nitrite was present as the sole nitrogen source. In contrast to *A. nidulans*, *M. grisea* was able to utilize L-aspartate or the glutamine-containing dipeptides as the sole nitrogen source in the presence of glufosinate, thus demonstrating metabolic differences between fungi, differences potentially relevant to the action and screening for inhibitory compounds.

PM also offers an additional advantage for eventual identification of new inhibitory components. Looking at the nutrient source utilization profile, it is evident that for every fungus or strain, there is a cluster of nutrients on which the organism grows even slower than on water and a number of nutrients enabling essentially the same growth rate as water. The observation of growth on water may be mostly due to the utilization of reserve carbohydrates and polyols present in the conidia (d′Enfert *et al*., 1999). Consequently, those nutrients, which result in an even slower growth, are likely inhibitors, at least, of the spore germination process. Examples of this are D-lactic acid methyl ester and glucuronamide for *H. jecorina*, which exhibit a statistically significant inhibitory effect (Druzhinina *et al*., 2006), and hydroxylamine for *A. nidulans* (Tanzer *et al*., 2003). While there may not be too much interest in developing inhibitory agents for *H. jecorina* and *A. nidulans*, similar strategies could be used to screen for biodegradable inhibitors against pathogenic plant and animal fungi.

Gardiner *et al*. (2009) used PMs to assess the role of different carbon and nitrogen sources that could be crucial for regulation of trichothecene synthesis by a plant pathogenic fungus, *Fusarium graminearum*, which causes a head blight disease of wheat. Presence of these toxins has important consequences for human and animal health. Gardiner *et al*. (2009) investigated the application of PMs in combination with a transgenic strain of *F. graminearum* in which the promoter from the TRI5 trichothecene biosynthesis gene was fused to green fluorescent protein (GFP). Using this strain in PM profiling, a variety of amines were identified, which significantly induce TRI5 expression.

### 2.8 Global inducer screening

The increase in available fungal genome sequences has also led to an increase in the identification of genes for which orthologues in even closely related species are unknown (Dogra and Breuil, 2004; OBrian *et al*., 2003; Schmoll *et al*., 2004). As explained in the introduction, studies of the genome-wide role of these genes are still limited to the use of mutants and their use in DNA-microarray analysis and proteomics (Sims *et al*., 2004). Identifying the physiological conditions under which a newly identified gene is expressed, however, is still restricted to the analysis of its transcript formation in individual experiments. Having an array-type system available for this purpose would greatly facilitate and accelerate the assignment of a function to new genes.

Our interest in this area comes from studies on chitinase formation by *Hypocrea atroviridis* (Mach *et al*., 1999; Brunner *et al*., 2003; Seidl *et al*., 2005). While there are several reports of chitinase gene expression in fungi in response to the availability of chitin or chitin degradation products (Carsolio *et al*., 1999; Mach *et al*., 1999; Donzelli and Harman, 2001; Kim *et al*., 2002), not all chitinases follow this rule. We have recently identified 18 chitinases in *H. jecorina*, and several of them are in fact not induced by chitin (Seidl *et al*., 2005). In order to find conditions leading to their induction, we have constructed recombinant strains containing various promoter-reporter fusions, inoculated them on PM plates, and photometricaly quantified the formation of the reporter enzyme. In our study, we used the *A. niger* glucose oxidase encoding (*goxA*) gene, for which an orthologue is absent in *Hypocrea*, because the enzyme is secreted into the medium and can therefore be assayed without cell breakage (Mach *et al*., 1999). The glucose oxidase assay involves formation of a green color, however, which is difficult to measure in the presence of the violet formazan dye formed by the redox dye implemented in the Biolog FF MicroPlates. Therefore, other non-invasive strategies such as the use of various forms of the green fluorescent protein or the luciferase (March *et al*., 2003; Arnone *et al*., 2004; Piwnica-Worms *et al*., 2004; Gu *et al*., 2004) may be alternatives. Nevertheless, the use of the *goxA* reporter enables us to identify new chitinase inducers in *Hypocrea*/*Trichoderma* (Verena Seidl, personal communication).

## 3 Concluding remarks and outlook

In this article, we have reviewed recent applications of PM technology as an accessory tool for global and genome-wide investigations on fungi, and reported on early achievements in the area. Although, not all the potential uses of this technology have already been experimentally verified, we anticipate that this broad phenotypic testing will become a simple

standard practice once the method becomes more familiar to scientists working with industrially important or pathogenic fungi. For example, in the past it was impractical to assay more than a few phenotypes and therefore it was impossible to measure phenotypic drift. One still untested use of PM technology is therefore the ability to provide researchers with a practical means to characterize each fungal isolate. To this end, the establishment of a public database of carbon and nitrogen utilization profiles for fungi (wild-type strains and mutants) would be an important instrument in understanding the comparative biochemistry and physiology of these organisms, and aid in the selection of appropriate strains if new products or strain properties are desired.

An advantage of the PM system is that it is much easier to perform than gene expression analysis. It involves only the preparation of a standardized fungal inoculum and its pipetting into the microplates of the standard PM plate. By contrast, to obtain specific and useful data on genes, one needs to perform genetic engineering work to create the strains to be analyzed. At this moment, the commercial availability of a sufficient number of nutrient conditions for fungi is still limited. In theory, one can devise hundreds or thousands of growth conditions in which specific biological pathways or physiological functions are linked to hyphal growth, but only about 2500 different assays are available for bacteria and yeasts, and only half are available for fungi.

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## References

Antal, Z., Kredics, L., Dóczi, I., Manczinger, L., Kevei, F., Nagy, E., 2002. The physiological features of opportunistic *Trichoderma* strains. *Acta Microbiol. Immunol. Hung.*, **49**:393.

Arnone, M.I., Dmochowski, I.J., Gache, C., 2004. Using reporter genes to study *cis*-regulatory elements. *Methods Cell Biol.*, **74**:621-652. [doi:10.1016/S0091-679X(04)74025-X]

Arst, H.N.Jr., Cove, D.J., 1973. Nitrogen metabolite repression in *Aspergillus nidulans*. *Mol. Gen. Genet.*, **126**(2):111-141. [doi:10.1007/BF00330988]

Arst, H.N.Jr., Scazzocchio, C., 1975. Initiator constitutive mutation with an up-promoter effect in *Aspergillus nidulans*. *Nature*, **254**(5495):31-34. [doi:10.1038/254031a0]

Avalos, J., Geever, R.F., Case, M.E., 1989. Bialaphos resistance as a dominant selectable marker in *Neurospora crassa*. *Curr. Genet.*, **16**(5-6):369-372. [doi:10.1007/BF00340716]

Avery, R.K., 2004. Prophylactic strategies before solid-organ transplantation. *Curr. Opin. Infect. Dis.*, **17**(4):353-356. [doi:10.1097/01.qco.0000136936.13662.74]

Bailey, C., Arst, H.N.Jr., 1975. Carbon catabolite repression in *Aspergillus nidulans*. *Eur. J. Biochem.*, **51**(2):573-577. [doi:10.1111/j.1432-1033.1975.tb03958.x]

Barredo, J.L., Alvarez, E., Cantoral, J.M., Diez, B., Martin, J.F., 1988. Glucokinase-deficient mutant of *Penicillium chrysogenum* is derepressed in glucose catabolite regulation of both beta-galactosidase and penicillin biosynthesis. *Antimicrob. Agents Chemother.*, **32**(7):1061-1067.

Blayer, S., Woodley, J.M., Dawson, M.J., Lilly, M.D., 1999. Alkaline biocatalysis for the direct synthesis of *N*-acetyl-D-neuraminic acid (Neu5Ac) from *N*-acetyl-D-glucosamine (GlcNAc). *Biotechnol Bioeng.*, **66**(2):131-136. [doi:10.1002/(SICI)1097-0290(1999)66:2<131::AID-BIT6>3.0.CO;2-X]

Bochner, B.R., 1988. New methods aid microbial identification. *Bio/Technology*, **6**(7):756. [doi:10.1038/nbt0788-756]

Bochner, B.R., 1989. Sleuthing out bacterial identities. *Nature*, **339**(6220):157-158. [doi:10.1038/339157a0]

Bochner, B.R., 2003. New technologies to assess genotype-phenotype relationships. *Nature Rev. Genet.*, **4**(4):309-314. [doi:10.1038/nrg1046]

Bochner, B.R., Gadzinski, P., Panomitros, E., 2001. Phenotype MicroArrays for high-throughput phenotypic testing and assay of gene function. *Genome Res.*, **11**(7):1246-1255. [doi:10.1101/gr.186501]

Brunner, K., Peterbauer, C.K., Mach, R.L., Lorito, M., Zeilinger, S., Kubicek, C.P., 2003. The Nag1 *N*-acetylglucosaminidase of *Trichoderma atroviride* is essential for chitinase induction by chitin and of major relevance to biocontrol. *Curr. Genet.*, **43**(4):289-295. [doi:10.1007/s00294-003-0399-y]

Brunner, K., Omann, M., Pucher, M.E., Delic, M., Lehner, S., Domnanich, P., Kratochwill, K., Druzhinina, I., Zeilinger, S., 2008. *Trichoderma* G protein-coupled receptors: genome analysis and functional characterization of a cAMP receptor-like protein from *Trichoderma atroviride*. *Curr Genet.*, **54**(6):283-299. [doi:10.1007/s00294-008-0217-7]

Caddick, M.X., Peters, D., Platt, A., 1994. Nitrogen regulation in fungi. *Antonie Van Leeuwenhoek*, **65**(3):169-177. [doi:10.1007/BF00871943]

Campos-Herrero, M.I., Bordes, A., Perera, A., Ruiz, M.C., Fernandez, A., 1996. *Trichoderma koningii* peritonitis in a patient undergoing peritoneal dialysis. *Clin. Microbiol. Newslett.*, **18**(19):150-151. [doi:10.1016/0196-4399(96)83918-3]

Carsolio, C., Benhamou, N., Haran, S., Cortes, C., Gutierrez, A., Chet, I., Herrera-Estrella, A., 1999. Role of the *Trichoderma harzianum* endochitinase gene, *ech42*, in mycoparasitism. *Appl. Environ. Microbiol.*, **65**(3):929-935.

Casas-Flores, S., Rios-Momberg, M., Bibbins, M., Ponce-Noyola, P., Herrera-Estrella, A., 2004. BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. *Microbiology*, **150**(Pt 11):3561-3569. [doi:10.1099/mic.0.27346-0]

Chaveroche, M.K., Ghigo, J.M., d'Enfert, C., 2000. A rapid method for efficient gene replacement in the filamentous fungus *Aspergillus nidulans*. *Nucleic Acids Res.*, **28**(22): E97. [doi:10.1093/nar/28.22.e97]

dela Cruz, T.E.E., Schulz, B.E., Kubicek, C.P., Druzhinina, I.S., 2006. Carbon source utilization by themarine *Dendryphiella* species *D. arenaria* and *D. salina*. *FEMS Microbiol. Ecol.*, **58**(3):343-353. [doi:10.1111/j.1574-6941.2006.00184.x]

Dogra, N., Breuil, C., 2004. Suppressive subtractive hybridization and differential screening identified genes differentially expressed in yeast and mycelial forms of *Ophiostoma piceae*. *FEMS Microbiol. Lett.*, **238**(1):175-181. [doi:10.1111/j.1574-6968.2004.tb09753.x]

Donzelli, B.G., Harman, G.E., 2001. Interaction of ammonium, glucose, and chitin regulates the expression of cell wall-degrading enzymes in *Trichoderma atroviride* strain P1. *Appl. Environ. Microbiol.*, **67**(12):5643-5647. [doi:10.1128/AEM.67.12.5643-5647.2001]

Dowzer, C.E.A., Kelly, J.M., 1991. Analysis of the *creA* gene, a regulator of carbon catabolite repression. *Mol. Cell. Biol.*, **11**(11):5701-5709.

Druzhinina, I.S., Schmoll, M., Seiboth, B., Kubicek, C.P., 2006. Global carbon utilization profiles of wild-type strains, mutants and transformants of *Hypocrea jecorina*. *Appl. Environ. Microbiol.*, **72**(3):2126-2133. [doi:10.1128/AEM.72.3.2126-2133.2006]

Druzhinina, I.S., Komon-Zelazowska, M., Kredics, L., Hatvani, L., Antal, Z., Belayneh, T., Kubicek, C.P., 2008. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. *Microbiology*, **154**(Pt 11):3447-3459. [doi:10.1099/mic.0.2008/021196-0]

d'Enfert, C., Bonini, B.M., Zapella, P.D., Fontaine, T., da Silva, A.M., Terenzi, H.F., 1999. Neutral trehalases catalyse intracellular trehalose breakdown in the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa*. *Mol. Microbiol.*, **32**(3):471-483. [doi:10.1046/j.1365-2958.1999.01327.x]

Farkaš, V., Labudová, I., Bauer, Š., Ferenczy, L., 1981. Preparation of mutants of *Trichoderma viride* with increased production of cellulase. *Folia Microbiol.*, **26**(2):129-132. [doi:10.1007/BF02927368]

Felenbok, B., Flipphi, M., Nikolaev, I., 2001. Ethanol catabolism in *Aspergillus nidulans*: a model system for studying gene regulation. *Prog. Nucleic Acid Res. Mol. Biol.*, **69**:149-204. [doi:10.1016/S0079-6603(01)69047-0]

Fodor, S.A., Rava, R.P., Huang, X.C., Pease, A.C., Holmes, C.P., Adams, C.L., 1993. Multiplexed biochemical assays with biological chips. *Nature*, **364**(6437):555-556. [doi:10.1038/364555a0]

Franzusoff, A., Cirillo, V.P., 1982. Uptake and phosphorylation of 2-deoxy-D-glucose by wild type and single-kinase strains of *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta*, **688**(2):295-304. [doi:10.1016/0005-2736(82)90340-6]

Friedl, M.A., Kubicek, C.P., Druzhinina, I.S., 2008a. Carbon source dependence and photostimulation of conidiation in *Hypocrea atroviridis*. *Appl. Environ. Microbiol.*, **74**(1): 245-250. [doi:10.1128/AEM.02068-07]

Friedl, M.A., Schmoll, M., Kubicek, C.P., Druzhinina, I.S., 2008b. Photostimulation of *Hypocrea atroviridis* growth occurs due to a cross-talk of carbon metabolism, blue light receptors and response to oxidativestress. *Microbiology*, **154**(Pt 4):1229-1241.

Gardiner, D.M., Kazan, K., Manners, J.M., 2009. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. *Fungal Genet. Biol.*, **46**(8):604-613. [doi:10.1016/j.fgb.2009.04.004]

Gu, M.B., Mitchell, R.J., Kim, B.C., 2004. Whole-cell-based biosensors for environmental biomonitoring and application. *Adv. Biochem. Eng. Biotechnol.*, **87**:269-305.

Guarro, J., Antolin-Ayala, M.I., Gene, J., Gutierrez-Calzada, J., Nieves-Diez, C., Ortoneda, M., 1999. Fatal case of *Trichoderma harzianum* infection in a renal transplant recipient. *J. Clin. Microbiol.*, **37**(11):3751-3755.

Hamer, L., Adachi, K., Montenegro-Chamorro, M.V., Tanzer, M.M., Mahanty, S.K., Darveaux, B.A., Lampe, D.J., Slater, T.M., Ramamurthy, L., DeZwaan, T.M., *et al*., 2001. Gene discovery and gene function assignment in filamentous fungi. *Proc. Natl. Acad. Sci. USA*, **98**(9): 5110-5115. [doi:10.1073/pnas.091094198]

Hennequin, C., Chouaki, T., Pichon, J.C., Strunski, V., Raccurt, C., 2000. Otitis externa due to *Trichoderma longibrachiatum*. *Eur. J. Clin. Microbiol. Infect. Dis.*, **19**(8): 641-642. [doi:10.1007/s100960000326]

Hoyos-Carvajal, L., Orduz, S., Bissett, J., 2009. Genetic and metabolic biodiversity of *Trichoderma* from Colombia and adjacent neotropic regions. *Fungal Genet. Biol.*, **46**(9):615-631. [doi:10.1016/j.fgb.2009.04.006]

Hynes, M.J., 1975. Studies on the role of the *areA* gene in the regulation of nitrogen catabolism in *Aspergillus nidulans*. *Aust. J. Biol. Sci.*, **28**(3):301-313.

Ilmén, M., Thrane, C., Penttilä, M., 1996. The glucose repressor gene *cre1* of *Trichoderma*: isolation and expression of a full-length and a truncated mutant form. *Mol. Gen. Genet.*, **251**(4):451-460. [doi:10.1007/s004380050189]

Jekosch, K., Kück, U., 2000. Glucose dependent transcriptional expression of the *cre1* gene in *Acremonium chrysogenum* strains showing different levels of cephalosporin C production. *Curr. Genet.*, **37**(6):388-395.

[doi:10.1007/s002940000121]

Kahmann, R., Basse, C., 2001. Fungal gene expression during pathogenesis-related development and host plant colonization. *Curr. Opin. Microbiol.*, **4**(4):374-380. [doi:10.1016/S1369-5274(00)00220-4]

Keppler, O.T., Horstkorte, R., Pawlita, M., Schmidt, C., Reutter, W., 2001. Biochemical engineering of the *N*-acyl side chain of sialic acid: biological implications. *Glycobiology*, **11**(2):11R-18R. [doi:10.1093/glycob/11.2.11R]

Kim, D.J., Baek, J.M., Uribe, P., Kenerley, C.M., Cook, D.R., 2002. Cloning and characterization of multiple glycosyl hydrolase genes from *Trichoderma virens*. *Curr. Genet.*, **40**(6):374-384. [doi:10.1007/s00294-001-0267-6]

Komon-Zelazowska, M., Bissett, J., Zafari, D., Hatvani, L., Manczinger, L., Woo, S., Lorito, M., Kredics, L., Kubicek, C.P., Druzhinina, I.S., 2007. Genetically closely related but phenotypically divergent *Trichoderma* species cause world-wide green mould disease in oyster mushroom farms. *Appl. Environ. Microbiol.*, **73**(22):7415-7426. [doi:10.1128/AEM.01059-07]

Kowal, P., Wang, P.G., 2002. UDP-GlcNAc C4 epimerase involved in the biosynthesis of 2-acetamino-2-deoxy-L-altruronic acid in the O-antigen repeating units of *Plesiomonas shigelloides* O17. *Biochemistry*, **41**(51):15410-15414. [doi:10.1021/bi026384i]

Kraus, G., Druzhinina, I., Bissett, J., Prillinger, H.J., Szakacs, G., Koptchinski, A., Gams, W., Kubicek, C.P., 2004. *Trichoderma brevicompactum* sp. nov. *Mycologia*, **96**(5):1059-1073. [doi:10.2307/3762089]

Kubicek, C.P., 1987. Involvement of a conidial endoglucanase and a plasma-membrane bound β-glucosidase in the induction of endoglucanase synthesis by cellulose in *Trichoderma reesei*. *J. Gen. Microbiol.*, **133**(6):1481-1487.

Kubicek, C.P., Bissett, J., Kullnig-Gradinger, C.M., Druzhinina, I.S., Szakacs, G., 2003. Genetic and metabolic diversity of *Trichoderma*: a case study on South-East Asian isolates. *Fungal Genet. Biol.*, **38**(3):310-317. [doi:10.1016/S1087-1845(02)00583-2]

Kudla, B., Caddick, M.X., Langdon, T., Martinez-Rossi, N.M., Bennett, C.F., Sibley, S., Davies, R.W., Arst, H.N.Jr., 1990. The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. *EMBO J.*, **9**(5):1355-1364.

Kutlesa, N.J., Caveney, S., 2001. Insecticidal activity of glufosinate through glutamine depletion in a caterpillar. *Pest Manage. Sci.*, **57**(1):25-32. [doi:10.1002/1526-4998(200101)57:1<25::AID-PS272>3.0.CO;2-I]

le Crom, S., Schackwitz, W., Pennacchio, L., Magnuson, J.K., Culley, D.E., Collett, J.R., Martin, J.R., Druzhinina, I.S., Mathis, H., Monot, F., *et al*., 2009. Tracking the roots of cellulase hyperproduction by the fungus *Trichoderma reesei* using massively parallel DNA sequencing. *PNAS*, **106**(38):16151-16156. [doi:10.1073/pnas.0905848106]

Mach, R.L., Peterbauer, C.K., Payer, K., Jaksits, S., Woo, S.L., Zeilinger, S., Kullnig, C.M., Lorito, M., Kubicek, C.P., 1999. Expression of two major chitinase genes of *Trichoderma atroviride* (*T. harzianum* P1) is triggered by different regulatory signals. *Appl. Environ. Microbiol.*, **65**(5):1858-1863.

March, J.C., Rao, G., Bentley, W.E., 2003. Biotechnological applications of green fluorescent protein. *Appl. Microbiol. Biotechnol.*, **62**(4):303-315. [doi:10.1007/s00253-003-1339-y]

Marzluf, G.A., 1997. Genetic regulation of nitrogen metabolism in the fungi. *Microbiol. Mol. Biol. Rev.*, **61**(1):17-32.

Montenecourt, B.S., Eveleigh, D.E., 1979. Selective Screening Methods for the Isolation of High Yielding Cellulase Mutants of *Trichoderma reesei*. *In*: Brown, R.D.Jr., Jurasek, L. (Eds.), Hydrolysis of Cellulose: Mechanisms of Enzymatic and Acid Catalysis. Advances in Chemistry, Vol. 181. American Chemical Society, p.289-301. [doi:10.1021/ba-1979-0181.ch014]

Nagy, V., Seidl, V., Szakacs, G., Komoń-Zelazowska, M., Kubicek, C.P., Druzhinina, I.S., 2007. Application of DNA bar codes for screening of industrially important fungi: the haplotype of *Trichoderma harzianum* sensu stricto indicates superior chitinase formation. *Appl. Environ. Microbiol.*, **73**(21):7048-7058. [doi:10.1128/AEM.00995-07]

Nogawa, M., Goto, M., Okada, H., Morikawa, Y., 2001. L-sorbose induces cellulase gene transcription in the cellulolytic fungus *Trichoderma reesei*. *Curr. Genet.*, **38**(6):329-334. [doi:10.1007/s002940000165]

OBrian, G.R., Fakhoury, A.M., Payne, G.A., 2003. Identification of genes differentially expressed during aflatoxin biosynthesis in *Aspergillus flavus* and *Aspergillus parasiticus*. *Fungal Genet. Biol.*, **39**(2):118-127. [doi:10.1016/S1087-1845(03)00014-8]

O′Farrell, P.H., 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, **250**(10):4007-4021.

Pall, M.L., 1993. The use of ignite (Basta; glufosinate; phosphinothricin) to select transformants of bar-containing plasmids in *Neurospora crassa*. *Fungal Genet. Newslett.*, **40**:58.

Piwnica-Worms, D., Schuster, D.P., Garbow, J.R., 2004. Molecular imaging of host-pathogen interactions in intact small animals. *Cell. Microbiol.*, **6**(4):319-331. [doi:10.1111/j.1462-5822.2004.00379.x]

Platt, A., Langdon, T., Arst, H.N.Jr., Kirk, D., Tollervey, D., Mates Sanchez, J.M., Caddick, M.X., 1996. Nitrogen metabolite signalling involves the C-terminus and the GATA domain of the *Aspergillus* transcription factor AREA and the 3 untranslated region of its mRNA. *EMBO J.*, **15**(11):2791-2801.

Ragnaud, J.M., Marceau, C., Roche-Bezian, M.C., Wone, C., 1984. Infection peritoneale a *Trichoderma koningii* sur dialyse peritoneale continue ambulatoire. *Med. Maladies Infect.*, **14**(7-8):402-405. [doi:10.1016/S0399-077X(84)80067-0]

Randez-Gil, F., Prieto, J.A., Sanz, P., 1995. The expression of

a specific 2-deoxyglucose-6P phosphatase prevents catabolite repression mediated by 2-deoxyglucose in yeast. *Curr. Genet.*, **28**(2):101-107. [doi:10.1007/BF00315774]

Ravagnani, A., Gorfinkiel, L., Langdon, T., Diallinas, G., Adjadj, E., Demais, S., Gorton, D., Arst, H.N.Jr., Scazzocchio, C., 1997. Subtle hydrophobic interactions between the seventh residue of the zinc finger loop and the first base of an HGATAR sequence determine promoter-specific recognition by the *Aspergillus nidulans* GATA factor AreA. *EMBO J.*, **16**(13):3974-3986. [doi:10.1093/emboj/16.13.3974]

Rieger, K.J., Kaniak, A., Coppee, J.Y., Aljinovic, G., Baudin-Baillieu, A., Orlowska, G., Gromadka, R., Groudinsky, O., di Rago, J.P., Slonimski, P.P., 1997. Large-scale phenotypic analysis-the pilot project on yeast chromosome III. *Yeast*, **13**(16):1547-1562. [doi:10.1002/(SICI)1097-0061(199712)13:16<1547::AID-YEA230>3.3.CO;2-P]

Rieger, K.J., El-Alama, M., Stein, G., Bradshaw, C., Slonimski, P.P., Maundrell, K., 1999. Chemotyping of yeast mutants using robotics. *Yeast*, **15**(10B):973-986. [doi:10.1002/(SICI)1097-0061(199907)15:10B<973::AID-YEA402>3.0.CO;2-L]

Ross-Macdonald, P., Coelho, P.S.R., Roemer, T., Agarwal, S., Kumar, A., Jansen, R., Cheung, K.H., Sheehan, A., Symoniatis, D., Umansky, L., *et al*., 1999. Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature*, **402**(6760):413-418. [doi:10.1038/46558]

Sampathkumar, P., Paya, C.V., 2001. *Fusarium* infection after solid-organ transplantation. *Clin. Infect. Dis.*, **32**(8):1237-1240. [doi:10.1086/319753]

Schauer, R., 2000. Achievements and challenges of sialic acid research. *Glycoconj. J.*, **17**(7-9):485-499. [doi:10.1023/A:1011062223612]

Schmoll, M., Zeilinger, S., Mach, R.L., Kubicek, C.P., 2004. Cloning of genes expressed early during cellulase induction in *Hypocrea jecorina* by a rapid subtraction hybridization approach. *Fungal Genet. Biol.*, **41**(9):877-887. [doi:10.1016/j.fgb.2004.06.002]

Schuster, A., Kubicek, C.P., Friedl, M.A., Druzhinina, I.S., Schmoll, M., 2007. Impact of light on *Hypocrea jecorina* and the multiple cellular roles of ENVOY in this process. *BMC Genomics*, **8**(1):449. [doi:10.1186/1471-2164-8-449]

Seiboth, B., Hartl, L., Pail, M., Kubicek, C.P., 2003. D-xylose metabolism in *Hypocrea jecorina*: loss of the xylitol dehydrogenase step can be partially compensated for by *lad1*-encoded L-arabinitol-4-dehydrogenase. *Eukaryot. Cell*, **2**(5):867-875. [doi:10.1128/EC.2.5.867-875.2003]

Seiboth, B., Gamauf, C., Pail, M., Hartl, L., Kubicek, C.P., 2007. The D-xylose reductase of *Hypocrea jecorina* is the major aldose reductase in pentose and D-galactose catabolism and necessary for β-galactosidase and cellulase induction by lactose. *Mol. Microbiol.*, **66**(4):890-900. [doi:10.1111/j.1365-2958.2007.05953.x]

Seidl, V., Huemer, B., Seiboth, B., Kubicek, C.P., 2005. A complete survey of *Trichoderma* chitinases reveals three distinct subgroups of family 18 chitinases. *FEBS J.*, **272**(22):5923-5939. [doi:10.1111/j.1742-4658.2005.04994.x]

Seidl, V., Druzhinina, I.S., Kubicek, C.P., 2006. A screening system for carbon sources enhancing β-*N*-acetylglucosaminidase formation in *Hypocrea atroviridis* (*Trichoderma atroviride*). *Microbiology*, **152**(Pt 7):2003-2012. [doi:10.1099/mic.0.28897-0]

Seidl, V., Gamauf, C., Druzhinina, I.S., Seiboth, B., Hartl, L., Kubicek, C.P., 2008. The *Hypocrea jecorina* (*Trichoderma reesei*) hypercellulolytic mutant RUT C30 lacks a 85 kb (29 gene-encoding) region of the wild-type genome. *BMC Genomics*, **9**(1):327. [doi:10.1186/1471-2164-9-327]

Sims, A.H., Gent, M.E., Robson, G.D., Dunn-Coleman, N.S., Oliver, S.G., 2004. Combining transcriptome data with genomic and cDNA sequence alignments to make confident functional assignments for *Aspergillus nidulans* genes. *Mycol. Res.*, **108**(Pt 8):853-857. [doi:10.1017/S095375620400067X]

Singh, M.P., 2009. Application of Biolog FF MicroPlate for substrate utilization and metabolite profiling of closely related fungi. *J. Microbiol. Methods*, **77**(1):102-108. [doi:10.1016/j.mimet.2009.01.014]

Strauss, J., Mach, R.L., Zeilinger, S., Hartler, G., Stöffler, G., Wolschek, M., Kubicek, C.P., 1995. Cre1, the carbon catabolite repressor protein from *Trichoderma reesei*. *FEBS Lett.*, **376**(1-2):103-107. [doi:10.1016/0014-5793(95)01255-5]

Sweigard, J.A., Carroll, A.M., Valent, B., 1999. Restriction Enzyme-mediated Integration in the Rice Blast Fungus. *In*: *Septoria* on Cereals: A Study in Pathosystems. IACR 15th International Symposium. Long Ashton Research Station, Bristol, p.192-198.

Tanis, B.C., van der Pijl, H., van Ogtrop, M.L., Kibbelaar, R.E., Chang, P.C., 1995. Fatal fungal peritonitis by *Trichoderma longibrachiatum* complicating peritoneal dialysis. *Nephrol. Dial. Transplant.*, **10**(1):114-116.

Tanzer, M.M., Arst, H.N., Skalchunes, A.R., Coffin, M., Darveaux, B.A., Heiniger, R.W., Shuster, J.R., 2003. Global nutritional profiling for mutant and chemical mode-of-action analysis in filamentous fungi. *Funct. Integr. Genomics*, **3**(4):160-170. [doi:10.1007/s10142-003-0089-3]

Vitale, R.G., Afeltra, J., Dannaoui, E., 2005. Antifungal combinations. *Methods Mol. Med.*, **118**:143-152.

Wilson, R.A., Arst, H.N.Jr., 1998. Mutational analysis of AREA, a transcriptional activator mediating nitrogen metabolite repression in *Aspergillus nidulans* and a member of the streetwise GATA family of transcription factors. *Microbiol. Mol. Biol. Rev.*, **62**(3):586-596.

Yeo, S.F., Wong, B., 2002. Current status of nonculture methods for diagnosis of invasive fungal infections. *Clin. Microbiol. Rev.*, **15**(3):465-484. [doi:10.1128/CMR.15.3.465-484.2002]

## Appendix

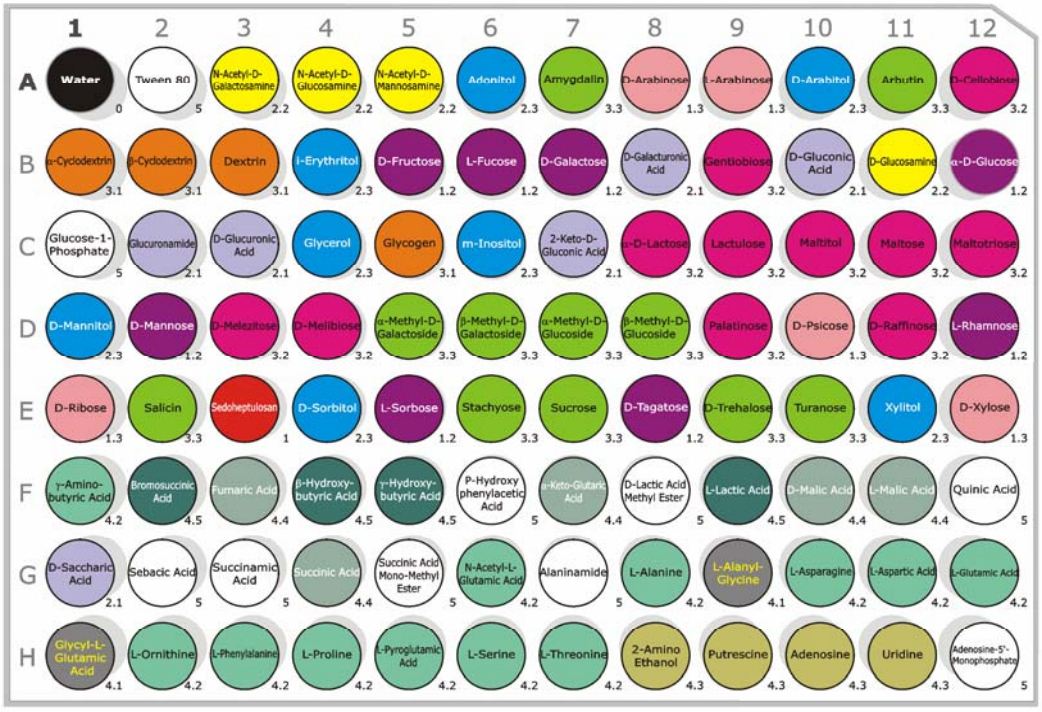


**Fig. 1A Carbon sources of Biolog FF MicroPlate**

Numbers indicate physiological and biochemical groups: 0: control; 1: monosaccharides: 1.1 heptose, 1.2 hexoses, 1.3 pentoses; 2: monosaccharide-related compounds: 2.1 sugar acids, 2.2 hexosamines, 2.3 polyols; 3: other sugars: 3.1 polysaccharides, 3.2 oligosaccharides, 3.3 glucosides; 4: nitrogen-containing compounds: 4.1 peptides, 4.2 L-amino acids, 4.3 biogene and heterocyclic amines, 4.4 TCA-cycle intermediates, 4.5 aliphatic organic acids; 5: others

**Introducing editorial board member:** Irina S. Druzhinina, the corresponding author of this invited review, is an editorial board member of *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*. Her study is focused on phylogeny and molecular ecology of Hypocreales fungi and on the development of bioinformatic tools for molecular identification of microbes. In the other line of research she is interested in metabolomics of filamentous fungi applying Phenotype MicroArray techniques. In 2001, she gained the doctorate of natural sciences of University of Vienna (Austria). Shortly after, she founded a research group on fungal evolution and functional biodiversity in Vienna University of Technology (Austria) and obtained a position of Assistant Professor in Department of Applied Biochemistry and Gene Technology.

Irina S. Druzhinina

Lea Atanasova, the first author of this invited review, studied biology and ecology at the University of Ljubljana (Slovenia) and has graduated from the University Vienna (Austria). She was awarded for her master thesis by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management as well as by Ecological Society of Germany, Austria and Switzerland. Soon after graduation, she joined the Irina S. Druzhinina's research group of fungal biodiversity and evolution in Vienna University of Technology (Austria) and started to work on her PhD thesis dedicated to the molecular ecology of soil mycobiota.

Lea Atanasova