Phase transition of a dimorphic fungus
*Penicillium marneffei*

Milena KORDALEWSKA, Dorota DRAPALÁ*

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**Abstract:** *Penicillium marneffei* is a pathogenic fungus that afflicts immune compromised individuals who live or travel in Southeast Asia. This species is unique, because it is the only dimorphic member of the genus *Penicillium*. Dimorphism results from a process, termed phase transition, which is regulated by temperature of incubation. At room temperature, the fungus grows filamentously (mould phase), but at body temperature (37°C), a uninucleate yeast form develops that reproduces by fission. Formation of the yeast phase appears to be a requisite for pathogenicity. The dimorphic nature of *P. marneffei* has attracted investigators interested in not only understanding the underlying molecular mechanisms of its unique mode of cellular development, but also in the identification of novel targets that might be exploited in the development of new chemotherapeutic modalities.

1. Introduction

The members of the genus *Penicillium* are filamentous fungi (one exception is thermally dimorphic *Penicillium marneffei*). *Penicillium spp.* are ubiquitous fungi - they are found in soil, decaying vegetation and in the air. *P. marneffei* is endemic specifically in Southeast Asia (Thailand and adjacent countries, Taiwan, and India) where it infects bamboo rats which serve as epidemiological markers and reservoirs for human infections. *Penicillium spp.* other than *P. marneffei* are commonly considered to be contaminants but may cause infections, particularly in immunocompromised hosts. *P. marneffei* is pathogenic particularly in patients with AIDS and its isolation from blood is regarded as an HIV marker in endemic areas (WHO, 2007). *P. marneffei* infections have also been reported in non-AIDS patients with haematological malignancies and those receiving immunosuppressive therapy (Wong et al., 2001). *P. marneffei* infection is acquired via inhalation and results in initial pulmonary infection, followed by fungaemia and dissemination of the infection. The lymphatic system, liver, spleen and bones are usually involved. Acne-like skin papules on the face, trunk, and extremities are observed during the course of the disease. The infections are often fatal (Cheng et al., 1998; Garbino et al., 2001; Rimek, 1999).

*Microbiology Department, Faculty of Chemistry, Gdańsk University of Technology*
Penicillium marneffei is the only known Penicillium species that exhibits temperature-dependent dimorphic growth. At 25°C, it grows in a mycelial form with vegetative conidia. Once conidia are inhaled, they undergo dimorphic switching to produce yeast cells at 37°C, the core temperature of humans. Thus, pathogenicity appears to be intimately linked to dimorphic transition. But the mechanism that regulates this switching remains a mystery (Chandler et al., 2008; Kummasook et al., 2011; Liu et al., 2007).

Fig. 1. Thermal dimorphism of P. marneffei A) The mould phase of P. marneffei (slide culture incubated at 25°C. B) The yeast phase of P. marneffei (produced from conidia incubated for 96 hours at 37°C) (Chandler et al., 2008)

The dimorphic nature of P. marneffei has attracted investigators interested in not only understanding the underlying molecular mechanisms of its unique mode of cellular development, but also in the identification of novel targets that might be exploited in the development of new chemotherapeutic modalities. Initial studies were limited to the morphological features of clinical isolates and the cellular events in the transition of the mould phase to the yeast phase (Chan and Chow, 1990; Garrison and Boyd, 1973; Pracharktam et al., 1992).

2. Differential gene expression

Discovering the regulation of Penicillium marneffei phase-specific genes might elucidate the control of its morphogenesis. Several genes that are expressed predominantly in the yeast form of Penicillium marneffei have been identified. Malate synthase-encoding gene was found to up-regulate in the yeast phase of Penicillium marneffei as an alternative energy producing pathway (Cooper Jr and Haycocks, 2000). Pongpomp et al. (2005) isolated and characterized a catalase-peroxidase protein-encoding gene (cpeA), which was expressed at a high level during yeast phase formation. Other genes possibly involved in regulating the dimorphic transition were also studied, e.g. abaA (Borneman et al., 2000), cflA (Boyce et al., 2001) and Tupa (Todd et al., 2003): the former appears to control the coupling of nuclear and cellular division in prearthroconidial cells, while the latter two control the correct morphogenesis of yeast cells. However, none of these have a detectable effect on dimorphic transition (Liu et al., 2007).
Investigation of differential gene expression during transition from the mycelial form to the yeast form of *P. marneffei* may lead to the discovery of candidate genes for pathogenicity. By application of suppression subtractive hybridization combined with real-time semi-quantitative RT-PCR, Liu et al. (2007) identified 43 differentially expressed genes. These genes were sorted into broad functional categories including cell wall synthesis, signal transduction, cell cycle, substance transport, general metabolism and stress response, etc. This suggests that a very complex series of molecular mechanisms is involved in the switching process of *P. marneffei* (Liu et al., 2007).

3. Actin expression

Actins are conserved eukaryotic proteins present mainly in the cell cytoplasm. They are essential cytoskeletal components of all fungi, involved in several cellular processes. Actin-encoding genes from a number of pathogenic fungi have been isolated and characterized.

In the study by Kummasook et al. (2011), the structure of the actin-encoding gene of *P. marneffei* was identified at both the nucleotide and amino acid levels. The number of nucleotides and amino acids for an open reading frame of actA in *P. marneffei* is similar to that of other fungal actins. Moreover, actA contains all the recognized conserved sequence motifs of actin protein sequences. The expression of actin genes has been studied in several pathogenic, dimorphic fungi. In *Sporothrix schenckii* and *Histoplasma capsulatum*, studies have demonstrated that the levels of actin gene transcripts vary during phase transition (el Rady and Shearer Jr, 1997; Han-Yaku, 1996). Similarly, in *Paracoccidioides brasiliensis* actin transcripts were found to be prevalent in the yeast form, but decreased during the transition from yeast to mold (Niño Vega et al., 2007).

The results of the study by Kummasook et al. (2011) suggest that the actin-encoding gene in *P. marneffei*—actA, increases in expression during the initial stages of morphogenesis and phase transition, but subsequently is transcribed at a steady state during normal mycelial and yeast growth. This relatively stable level of expression appears to be independent of the stress response in *P. marneffei*. Hence, actA transcription appears to be a suitable standard to serve as an internal control during quantitative analysis of gene expression in *P. marneffei* for a short period of incubation (30 min) under stress conditions and for longer incubation periods (2–4 h) during macrophage infection with conidia (Kummasook et al., 2011).

4. Protein profiling

As mentioned before, no specific genes have been identified in *P. marneffei* that strictly induce mould-to-yeast phase conversion. Chandler et al. generated protein profiles from the *P. marneffei* yeast and mould phases to help identify potential gene products associated with morphogenesis. Whole cell proteins from the early stages of *P. marneffei* mould and yeast development were resolved by two-dimensional gel electrophoresis. Selected proteins were recovered and sequenced by capillary-liquid chromatography-nanospray tandem mass spectrometry. Putative identifications were
derived by searching available databases for homologous fungal sequences. Proteins found common to both mould and yeast phases included the signal transduction proteins cyclophilin and a RACK1-like ortholog, as well as those related to general metabolism, energy production, and protection from oxygen radicals. Many of the mould-specific proteins identified possessed similar functions. By comparison, proteins exhibiting increased expression during development of the parasitic yeast phase comprised those involved in heat-shock responses, general metabolism, and cell-wall biosynthesis, as well as a small GTPase that regulates nuclear membrane transport and mitotic processes in fungi. The cognate gene encoding the latter protein, designated RanA, was subsequently cloned and characterized. The *P. marneffei* RanA protein sequence, which contained the signature motif of Ran-GTPases, exhibited 90% homology to homologous *Aspergillus* proteins (Chandler et al., 2008).

The study by Chandler et al. (2008) demonstrated the utility of proteomic approaches to studying dimorphism in *P. marneffei*. Moreover, this strategy complements and extends current genetic methodologies directed towards understanding the molecular mechanisms of phase transition. Finally, the documented increased levels of RanA expression suggest that cellular development in *P. marneffei* involves additional signaling mechanisms than have been previously described (Chandler et al., 2008).

References


Liu, H., L. Xi, J. Zhang, X. Li, X. Liu, C. Lu and J. Sun (2007), ‘Identifying differen-


