From Bamboo Rats to Humans: the Odyssey of *Penicillium marneffei*

At first a mere peculiarity, this fungal infection is now a serious AIDS-associated public health problem in Southeast Asia

Chester R. Cooper, Jr.

In 1955, the spontaneous deaths of three bamboo rats caught the attention of investigators associated with the Pasteur Institute in South Vietnam. These rats, belonging to the native species *Rhizomys sinensis*, were being used to study rickettsial diseases. However, although the rats died from a fulminant infection of the reticuloendothelial system caused by a yeast-like agent, the fungus isolated from the rats was a species of *Penicillium*, a genus whose members grow as molds.

The recovery of this fungus was unusual because *Penicillium* species were not considered pathogens of humans or animals. Hence, these curious investigators sent both the fungus and a laboratory mouse inoculated with the putative pathogen to Gabriel Segretain, a colleague at the Pasteur Institute in Paris. Segretain identified the fungus as a new species and named it *Penicillium marneffei* in honor of Hubert Marneffe, director of the Pasteur Institute in Vietnam.

The *P. marneffei* story that began with these incidents is illustrative of the surprising and unexpected infectious agents emerging from tropical regions. From its obscure beginnings as a disease of bamboo rats in Southeast Asia to its explosive rise as a major pathogen among AIDS patients, the fungus typifies how seemingly innocuous organisms adapt to changing patterns in world demographics, health care, industrialization, travel, immigration, and human behavior.

The threat from *P. marneffei* to public health continues to grow, bringing this once-inconspicuous fungus to the attention of clinicians serving populations in regions outside the area in which it is endemic. Undoubtedly, *P. marneffei* will become a greater challenge, judging from the recent United Nations report indicating that more than 16,000 individuals per day are being infected with human immunodeficiency virus (HIV), most in developing countries. Information gathered from studying this pathogen can be used to battle *P. marneffei* and interrupt its trek, but it also serves to address similar tropical pathogens.

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Early on, Infections a Peculiarity, Not a Public Health Threat

In 1959, Segretain accidentally pricked his finger with a needle he was using to infect hamsters with *P. marneffei*. Within two weeks, a localized lesion appeared at the site of his injury. Segretain subsequently experienced lymphangitis and lymph node hypertrophy. Fortunately, excision of the lesion followed by intensive therapy with nystatin eliminated the fungal infection. However, the inauspicious odyssey of an emerging fungal pathogen had begun in which the dubious, and perhaps embarrassing, honor of being the first known human infected with *P. marneffei* fell to Segretain.

Despite Segretain's infection, *P. marneffei* appeared more a peculiarity than a threat to human health. Not until 1973 was the first natural human infection reported. The patient, a 61-year-old male U.S. citizen who had previously traveled to Southeast Asia, was diagnosed during treatment for Hodgkin's disease. More than a decade elapsed before a second case was reported in 1984. Again, the disease was diagnosed in a male U.S. citizen of about the same age who had traveled throughout the Far East. Both patients survived for several years follow-

ing their initial diagnosis and died from conditions other than infection (penicilliosis) due to *P. marneffei*.

Also in 1984, seven similar cases of *P. marneffei* infections in Bangkok, Thailand, were reported, with five of these cases being diagnosed between 1972 and 1982. In 1985, eight more cases that occurred in China between 1964 and 1983 were retrospectively diagnosed as *P. marneffei* infections after having been initially ascribed to histoplasmosis. Through 1988, at least 30 cases had been reported throughout Southeast Asia as well as in countries outside that region. Only 10 of these patients were found to have underlying predisposing conditions unrelated to AIDS.

By 1988, however, *P. marneffei* infections became more than just a miscellaneous oddity in the medical mycology literature. The exploding HIV epidemic in Southeast Asia was fueling a volatile chain reaction of opportunistic infectious diseases potentially afflicting millions of people. Thus, in 1988, *P. marneffei* emerged as a serious public health threat, especially for individuals residing in northern Thailand.

AIDS and Penicilliosis Due to *P. marneffei*

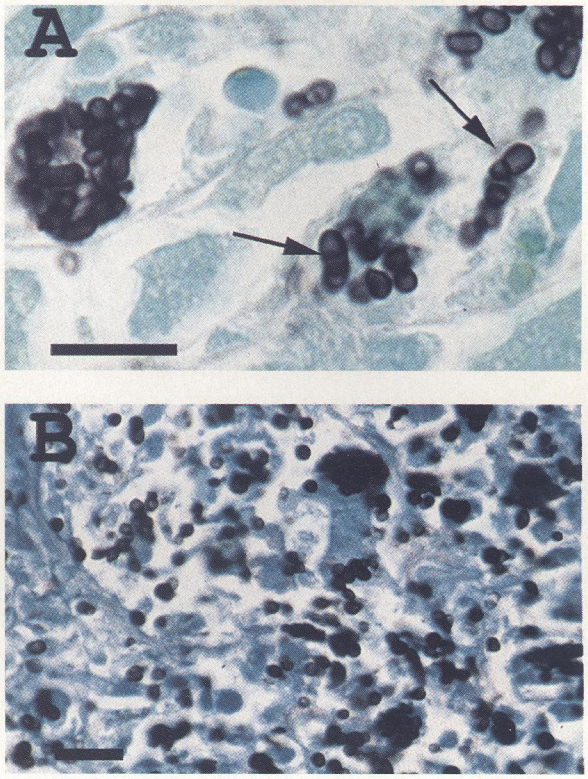
The first cases of *P. marneffei* infections among confirmed HIV-positive individuals were reported in 1988. Of the first 44 patients, 12 developed penicilliosis after returning to their native countries following visits to Southeast Asia. Members of this group included citizens of Australia, France, Italy, the Netherlands, the United Kingdom, and the United States. To date, at least 21 cases of pencilliosis due to *P. marneffei* have been described among non-Asian, HIV-infected individuals.

Another non-Asian case occurred in a Congolese HIV-positive physician who presumably became exposed to *P. marneffei* during a training session at the Pasteur Institute in Paris. Though the individual did not directly handle the fungus, other students in the building during this period were working with it. Air samples taken during this same time were negative for *P. marneffei*, suggesting that few airborne propagules are necessary to initiate symptomatic infections in immunocompromised hosts.

The greatest impact of this fungal disease has been felt by the indigenous population of Southeast Asia, particularly AIDS patients in the Chiang Mai province of Northern Thailand. By 1992, penicilliosis due to *P. marneffei* was declared an AIDS-related indicator disease in Southeast Asia. In the same year, one hospital in Chiang Mai alone reported 86 cases of penicilliosis among 92 HIV-positive adults. According to a later survey of more than 300 AIDS patients in Chiang Mai, 16% of them had contracted pencilliosis, the third most frequent opportunistic infectious disease of this patient group, trailing only tuberculosis (31%) and cryptococcosis (25%).

As the number of AIDS cases in Thailand rose 13-fold between 1991 and 1994, more than 550 penicilliosis cases were recorded in the Chiang Mai. The following year, that number more than doubled to over 1,300. Moreover, according to a 1996 study of Chinese AIDS patients in Hong Kong, *P. marneffei* and cytomegalovirus infections resulted in the same number of deaths (7.5%) and were the most common cause of

FIGURE 1



The arthroconidia in the tissue phase of *P. marneffei* (A) divide by fission (arrows), whereas the yeast cells of *H. capsulatum* (B) reproduce by budding. The bar in each frame represents 10 μm.

mortality after *Pneumocystis carinii* (23.9%), *Cryptococcus neoformans* (10.4%), and *Mycobacterium avium* (8.9%).

The dramatic rise in the incidence of penicilliosis correlates with available evidence suggesting that a significant number of symptomless individuals are infected with *P. marneffei* and will eventually develop disseminated disease due to reactivation of the pathogen upon immunosuppression. Also, because predictions indicate that the AIDS epidemic will continue its explosive upsurge in Thailand, as well as in other Southeast Asian countries such as China, a proportionate increase in penicilliosis is likely.

P. marneffei infections remain a significant public health problem in the Far East. The relative ease of present-day intercontinental travel, the escalation in legal and illegal immigration, and the rising worldwide frequency of HIV infection make it likely that more infected individuals will be recognized among populations outside this geographic region, thereby providing new challenges in the battle against this AIDS-related infection.

The Role of the Bamboo Rat

The association of *P. marneffei* with bamboo rats has intrigued many investigators. No other animal besides humans is known to be naturally infected by this pathogen. How this relationship between rat and fungus might play a role in transmitting disease to humans has been the subject of several elaborate field studies.

Investigators reasonably postulated that *P. marneffei* is a natural part of the flora inhabiting vegetation and the soil. Thus, bamboo rats encounter the fungus as part of their natural habitat. Moreover, since bamboo rats are sighted frequently near human dwellings and serve as a food source for members of the indigenous human population, transmission of the fungus to susceptible individuals seems possible. Yet, what first appeared to be a simple relationship explaining the transmission of this fungus between rats and humans has become an enigma.

Table 1. Frequency of *P. marneffei* among captured bamboo rats[a]

Bamboo rat species	*P. marneffei* isolation frequency
Rhizomys sinensis	100 % (n = 5)
Rhizomys pruinosis	66.4% (n = 244)
Rhizomys sumatrensis	92.9% (n = 14)
Cannomys badius	9.8% (n = 92)

[a] From the collective data obtained during seven separate field studies conducted in Vietnam, China, and Thailand.

Two genera of bamboo rats are found in Southeast Asia. One genus, *Rhizomys*, contains at least three species: *R. sinensis*, *R. pruinosis*, and *R. sumatrensis*. The second genus, *Cannomys*, contains a single species, *C. badius*, which includes two distinct groups: the reddish-brown and greyish-black rats. Individual species of both genera are found in particular geographical regions, but collectively they cover an area from northern China and Nepal to Malay and Sumatra.

Three separate epidemiological studies conducted during the 1980s and published in English indicate that the predominant species of bamboo rat in southern China, *R. pruinosis*, carries *P. marneffei* in its internal organs. Collectively, 62 apparently healthy rats (60 *R. pruinosis* and 2 *R. sinensis*) were captured and sacrificed, with *P. marneffei* being recovered from the internal organs of 54 animals (87%). In one of these studies, *P. marneffei* was also isolated from the feces of three of four bamboo rats as well as from three of their burrows. An additional study published in Chinese described similar observations in which *P. marneffei* was recovered from 114 of 179 (64%) *R. pruinosis* rats.

Several more recent studies focus on the infection rate of bamboo rats from Thailand. One research group led by Li Ajello, formerly of the U.S. Centers for Disease Control and Prevention, captured bamboo rats from the central and southern regions of Thailand. Of the 39 otherwise undistinguished rats collected, 8 were *R. pruinosis* and 31 were *C. badius*. However, although 6 of the *R. pruinosis* rats carried *P. marneffei* in their internal organs, only 6 (19%) of *C. badius* rats were infected with *P. marneffei*. This apparently low infection rate among the *C. badius* rats was subsequently confirmed by Suwat Chariyalertsak and colleagues in their studies of bamboo rats captured in the Chiang Mai province of northern Thailand.

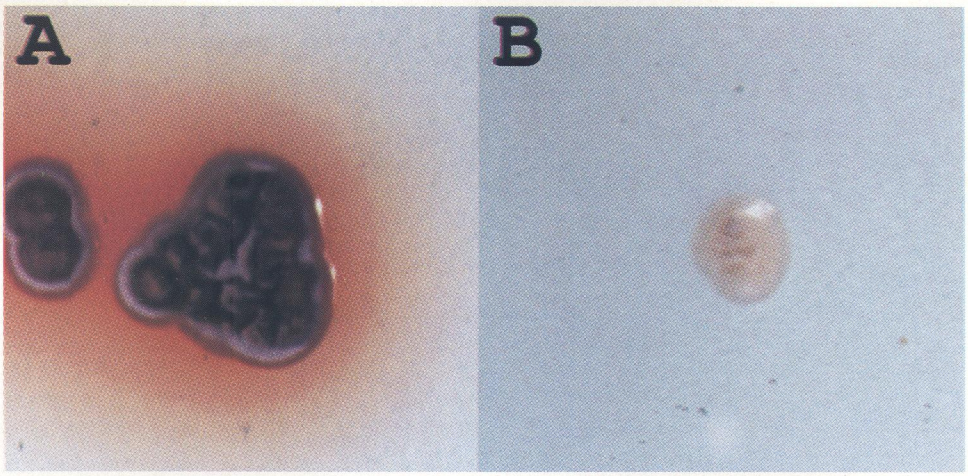
Together, these data from seven published studies show that the bamboo rat is often a carrier of this fungus (Table 1). Conceivably, the bamboo rat could serve as a vehicle for transmitting the disease to humans. However, in a recent case control study, Chariyalertsak and colleagues could not establish exposure to bamboo rats as a risk factor for acquiring penicilliosis, even though some people live in proximity to

rat habitats and consume them as food. They propose that exposure to soil, especially during the rainy season in the tropical climes of Thailand, is the critical risk factor associated with acquisition of *P. marneffei* infection.

That proposal is perplexing, because *P. marneffei* has rarely been isolated from soil or vegetation that surrounds rat burrows or the homes of infected individuals. In a previous study, these same investigators examined soil taken from 28 burrows of the bamboo rats as well as 67 soil and vegetation samples from near residential areas of individuals afflicted with *P. marneffei*. Only one sample, that being soil from a rat burrow, proved to be culture positive.

Exactly how and where both humans and bamboo rats acquire the organism is not clear. Perhaps the present situation is analogous to the relationship between sporotrichosis and sphagnum moss. Sphagnum moss derived from particular bogs in the northern United States is associated with several epidemics of *Sporothrix schenckii* infections. However, *S. schenckii* has never been isolated from the bogs or immediate surroundings. Only after the moss was dried and transported was the causative agent isolated. The source of contamination has never been located. Similarly, the exact environmental origin of *P. marneffei* remains a mystery.

FIGURE 2



The mold phase of *P. marneffei* produces a diffusible rose-colored pigment (A), whereas the arthroconidial phase does not (B).

Clinical Aspects of *P. marneffei* Infection

The common clinical manifestations of *P. marneffei* infections among both HIV-positive and HIV-negative patients include fever, chronic coughing, pulmonary infiltrates, generalized lymphadenopathy, septicemia, anemia, hepatomegaly, splenomeg-aly, weight loss, diarrhea, and skin lesions (Table 2). Despite the similar presentations, the onset of certain symptoms in HIV-positive patients is more intense and acute, especially among children. In contrast, bone lesions appear more frequently in non-AIDS patients. Of particular interest are the papulonecrotic skin lesions that form in both patient groups. These lesions are considered characteristic of penicilliosis caused by *P. marneffei*. However, they can be confused with similar skin lesions associated with tuberculosis, molluscum contagiosum, cryptococcosis, and histoplasmosis.

In vitro susceptibility testing indicates that isolates of *P. marneffei* are generally susceptible to agents commonly used to treat deep-seated mycoses. However, few studies have tried to correlate in vitro susceptibility data and outcome of infection. In perhaps the best-documented study, Khaunchai Supparatpinyo and colleagues found that 28 isolates of *P. marneffei* from 86 Thai HIV-infected adults were highly susceptible to itraconazole, ketoconazole, miconazole, and 5-fluorocytosine; moderately susceptible to amphotericin B; and generally resistant to fluconazole. These results paralleled the clinical outcome of patients in which treatment failure or relapse was greater with fluconazole treatment but significantly less in patients receiving amphotericin B or itraconazole.

In a separate study, pediatric HIV patients responded better to treatment than did adult patients. Among non-AIDS patients, published data suggest that 25% of adults and 50% of

Table 2. Clinical signs of *Penicillium marneffei* infection among HIV-positive and -negative patients[a]

	Percentage of patients exhibiting symptom[b]			
	HIV-positive		HIV-negative	
Symptoms	Adults (n = 112)	Children (n = 22)	Adults (n = 41)	Children (n = 6)
Fever	94.6	95.5	75.6	66.7
Weight Loss	69.6	NR[c]	12.2	16.7
Anemia	56.3	NR	22.0	16.7
Lymphadenopathy	55.4	86.4	58.5	33.3
Hepatomegaly	46.9	86.4	2.4	50.0
Splenomegaly	16.1	68.2	2.4	NR
Skin Lesions	65.2	63.6	68.3	NR
Diarrhea	15.8[d]	4.5	NR	16.7
Cough	40.4[d]	9.1	26.8	16.7

[a]Data taken from papers published in English and French through 1997.
[b]Males comprised 92% and 77% of the HIV-infected adults and children, respectively. Among HIV-negative individuals, 63% of the adults and 83% of the children were male.
[c]NR, not reported.
[d]Data from only 57 individuals.

children treated with antifungal agents respond positively or are cured. The collective evidence suggests that *P. marneffei* infections are most effectively treated initially with amphotericin B and that concurrent azole therapy may also be beneficial. AIDS patients typically receive a prophylactic maintenance regimen involving itraconazole, ketoconazole, or perhaps fluconazole after amphotericin B treatment.

Although the host response to *P. marneffei* infection is not well understood, cellular immunity plays a significant role. For instance, cellular immunity plays a role in mice that are infected with this microorganism. In several clinical cases of penicilliosis, restoration of host cellular defenses prompted a positive clinical response or elimination of the infection. Severe infection causes death via a CD4+ T cell-mediated hyperinflammatory reaction. Yet, the role of tumor necrosis factor alpha (TNF-alpha) as a proinflammatory stimulator in this process is ambiguous. Although anti-TNF-alpha antibody does not prolong survival, interleukin-12 does.

In vitro studies with cultured human cell lines also implicate several of these growth factors as part of the response to *P. marneffei*. For example, according to Stuart Levitz of the Boston University Medical Center, peripheral blood mononuclear cells from humans release significant amounts of TNF-alpha when stimulated by *P. marneffei* arthroconidia. Moreover, *P. marneffei* stimulates proliferation of the peripheral blood mononuclear cells. The lymphoproliferation presumably results from cross-reactive antigens, because the human donors had no travel history to endemic regions of Southeast Asia.

According to Massimo Cogliati and colleagues at the Università deglia Studi di Milano, Italy, nonstimulated murine macrophages are damaged following phagocytosis of *P. marneffei* conidia. However, stimulated macrophages reduce the number of intracellular arthroconidia and damage the fungus. The investigators speculate that the L-arginine-dependent nitric oxide pathway is involved in murine host cell defense, functioning in the same way as it does when exposed to other pathogenic fungi.

Diagnosis of Infection

Diagnosing *P. marneffei* infections can prove challenging. One complicating factor is that *Histoplasma capsulatum*, the causative agent of histoplasmosis, closely resembles *P. marneffei*. Moreover, both are intracellular parasites. Presumably, pathogenesis begins via the inhalation of infectious propagules, primarily conidia.

As in histoplasmosis, the initial host response to *P. marneffei* is histiocytic in nature. The

phagocytized conidia grow as globose-to-oval yeast-like cells, better described as arthroconidia, that measure slightly larger than *H. capsulatum* (Fig. 1). The *P. marneffei* arthroconidia reproduce by schizogony ("fission") and characteristically contain a single centrally located transverse septum. This differs from cells of *H. capsulatum*, which reproduce in vivo as budding yeasts.

To facilitate histopathologic recognition of *P. marneffei*, Leo Kaufman and coworkers at the Centers for Disease Control and Prevention developed a highly specific fluorescent antibody test. The antibody reacts only with the arthroconidial form of *P. marneffei*. Although other investigators have used a monoclonal antibody to detect *P. marneffei* in human tissues, the antibody is not specific. Originally developed to detect *Aspergillus* spp., it binds to a galactomannan epitope common to both this fungus and *P. marneffei*.

Other methods are being used to detect antibodies to *P. marneffei* in the serum of HIV-positive and HIV-negative individuals (Table 3). For instance, researchers from several separate groups have developed assays using yeast phase-specific 50- and 54-kilodalton proteins, yeast cytoplasmic antigens, non-phase-specific 38- and 90-kilodalton proteins, germinating conidia, mycelial culture filtrates, and arthroconidial culture filtrates as antigens for detecting antibodies to *P. marneffei* in human serum. Though apparently quite specific, the sensitivities of these tests vary depending upon the detection method used and the type of patient examined.

Similar assays using antigens isolated for detecting *H. capsulatum* or *Aspergillus* antibodies in serum cross-react with *P. marneffei* antigens. In contrast, Kaufman and colleagues developed immunodiffusion and latex agglutination tests to assess levels of serum antigens from *P. marneffei*. Individually, these tests are moderately sensitive. When used concurrently, though, the sensitivity of these tests is greater than 80%. Collectively, these antibody and antigen tests have a high potential diagnostic and prognostic value, but further practical developments are needed.

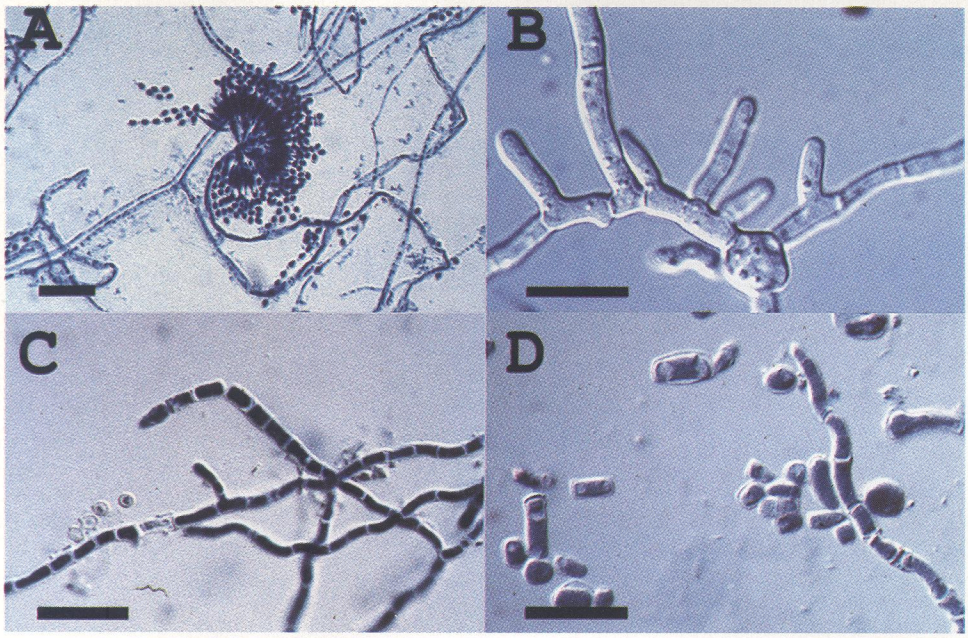
Table 3. Reported serological methods to detect *Penicillium marneffei* antibodies in human sera

Investigators	Year	Type of antigen	Assay used
Sekon et al.	1982	Mycelial culture filtrate	Immunodiffusion
Viviani et al.	1993	Mycelial culture filtrate	Immunodiffusion
Sekhon et al.	1994	Mycelial culture filtrate	Immunodiffusion
Yuen et al.	1996	Germinating conidia	Indirect immunofluorescence
Kaufmann et al.	1996	Arthroconidial culture filtrate	Immunodiffusion
Chongtrakool et al.	1997	38-kDa protein	Immunoblot
Imwidthaya et al.	1997	Mycelial culture filtrate	Microimmunodiffusion
Vanittanakom et al.	1997	50-kDa and 54-kDa arthroconidial phase proteins	Immunoblot
Jeavons et al.	1998	Cytoplasmic yeast antigens (50, 54, and 61 kDa)	Immunoblot
Cao et al.	1998	38- and 90-kDa mannoprotein	Immunoblot

The definitive method of diagnosis depends on culturing *P. marneffei* from clinical specimens. Cells can then be identified using an exoantigen test, which is not commercially available. This test is impractical for the typical clinical laboratory for other reasons. Most often, colonial and morphological features are used to identify *P. marneffei*. When a fungus is isolated, care must be taken to distinguish *P. marneffei* from other *Penicillium* species. The pink-to-red pigment produced at room temperature by *P. marneffei* is not strictly characteristic of this species (Fig. 2); other *Penicillium* species considered to be contaminants may also exhibit this feature.

The key diagnostic feature is the thermally induced production of arthroconidia in vitro. However, *Paecilomyces* strains, which resemble *Penicillium* species both in vitro and in vivo, can also cause infections in animals and compromised hosts. Nonetheless, these two genera can be readily distinguished from one

FIGURE 3



Conidia from the mold phase (A) of *P. marneffei* incubated at 37°C undergo phase transition by initially forming short, septate hyphae (B). Subsequently, the hyphae break apart at the septa (C) to form single-celled arthroconidia. These forms continue to grow and divide by fission, eventually comprising the majority of cells in the culture (D). The bar in each frame represents 10 μm.

another by examination of conidial morphology and ontogeny.

Dimorphism in *P. marneffei*

Although *P. marneffei* is dimorphic, only one published study describes the ultrastructural changes accompanying the shift from mold to arthroconidial phase. Because that study also discloses few details about mechanisms underlying these changes in cell shape, my colleagues and I began studying dimorphism in *P. marneffei*.

Early on, we recognized some parallels between *P. marneffei* and *H. capsulatum*. For example, the conversion or phase transition is thermally regulated in vitro. At 25–30°C, *P. marneffei* grows as a mold bearing the typical reproductive structure of members belonging to the genus *Penicillium* (Fig. 3A). After 6 to 12 hours at 37°C, however, individual conidia swell and begin a short period of isotropic growth. Subsequently, one or two loci at the surface of the conidium begin to bulge, developing apically into a septate, hyphal element (Fig. 3B).

Segments of varying length form that, as the hyphae age, appear to fragment along septal planes that can be characterized as double septa (Fig. 3C). This process generates single cells that tend to continue to reproduce by fission (Fig. 3D). The ontogeny of these cells defines them as arthroconidia rather than yeasts. As the culture ages, more and more arthroconidia form. This entire process is reversible, meaning that apical hyphal development and subsequent conidiogenesis occur when the arthroconidia are held at 25–30°C.

The reversible nature of phase transition in *P. marneffei* implies that cells do not become unalterably committed to a specific mode of cellular development. Rather, phase transitions may be strictly regulated by the expression of phase-specific genes. My colleagues and I have identified several candidate phase-specific genes from *P. marneffei* as part of a larger effort to better understand phase transition. Conceivably, some of these genes may represent virulence factors. Efforts are now underway to establish their role in phase transition as well as their potential role in virulence. By understanding the underlying mechanisms of phase transition, we hope to uncover critical information regarding the basis for fungal pathogenesis which may help us identify new target sites for the development of antifungal agents or immunomodulation therapies.

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