I am an incoming senior, pursuing a BS in Molecular Biosciences and Biotechnology and BA in Philippine Language and Literature. My highest academic goal is to receive a dual MD-PhD degree and become a Filipino-American physician scientist. My *Mānoa Horizons* entry is an international biomedical research collaboration between the University of Hawai'i John A. Burns School of Medicine and Chiang Mai University Faculty of Medicine in Thailand, supported by the Minority Health and Health Disparities International Research Training (MHIRT) grant from the National Institutes of Health (NIH). This research project has enabled me to reflect on the unique biomedical concerns that developing nations, like Thailand and the Philippines, have but are almost non-existent in developed nations like the United States. *Penicilliosis marneffei,* the human mycosis that is associated with the fungus I studied, is endemic to Southeast Asia, and affects immunocompromised HIV-positive and AIDS patients – patients whose counterparts are now being treated with highly active antiretroviral therapy (HAART) in developed nations. Many of the pathogenic microorganisms that cause human diseases have now been eradicated in developed nations, but these disease-causing microorganisms are still important biomedical research subjects because of their prevalence in developing nations.

# Time-Dependent Morphological Transformation of *Penicillium marneffei* by the Expression of Yeast-Phase Antigen in Liquid Culture and in THP-1 Cell Line

**McMillan Ching**

Summer International Research Training
*Mentor:* Dr. Axel Lehrer

Penicillium marneffei *is a thermally dimorphic fungal pathogen associated with HIV infection. It causes penicilliosis, the third most common AIDS-defining illness in northern Thailand. Due to the considerable interest on the dimorphism of* P. marneffei*, it is hypothesized that the time required for* P. marneffei *to transition from its mold form to its yeast-like form affects the virulence of the organism inside the mammalian host. This study investigated the expression of a yeast-specific antigen in phase transition of* P. marneffei *by using the yeast-specific monoclonal antibody(MAb) 4D1. The conidia of* P. marneffei *were inoculated in 1% Proteose followed by harvest at 12-hour time intervals from 24 to 144 hours. Cells were then incubated with MAb 4D1 followed by fluorescently labeled secondary antibody. The percentage of* P. marneffei *positive yeast cells, detected by flow cytometry, increased gradually after longer incubation, in the range of 2.5-51.8 % of cells. In addition, THP-1 cells, were infected with* P. marneffei *at MOI=2 from 12 to 60 hours. Cells were harvested at 12-hour time intervals followed by staining using Mab 4D1 as described above. It was observed that 23.8% of the infected cells were positive at 24 hours of incubation, increasing to 61.85% after 36 hours. The dimorphism of* P. marneffei *in THP-1 was more rapid than found in liquid culture of 1% Proteose. The faster transformation of yeast cells in mammalian host cells than in liquid cell culture may suggest it being linked to better survival and increased virulence inside human and other mammalian hosts.*

(See poster on next page)

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# Time-Dependent Morphological Transformation of *Penicillium marneffei* by the Expression of Yeast-Phase Antigen in Liquid Culture and in THP-1 Cell Line

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## ABSTRACT

*Penicillium marneffei* is a thermally dimorphic fungal pathogen associated with HIV infection. It causes penicilliosis, the third most common AIDS-defining illness in northern Thailand. Due to the considerable interest on the dimorphism of *P. marneffei*, it is hypothesized that the time required for *P. marneffei* to transition from its mold form to its yeast-like form affects the virulence of the organism inside the mammalian host. This study investigated the expression of a yeast-specific antigen in phase transition of *P. marneffei* by using the yeast-specific monoclonal antibody(MAb) 4D1. The conidia of *P. marneffei* were inoculated in 1% Protease followed by harvest at 12-hour time intervals from 24 to 144 hours. Cells were then incubated with MAb 4D1 followed by fluorescently labeled secondary antibody. The percentage of *P. marneffei* positive yeast cells, detected by flow cytometry, increased gradually after longer incubation, in the range of 2.5-51.8 % of cells. In addition, THP-1 cells, were infected with *P. marneffei* at MOI=2 from 12 to 60 hours. Cells were harvested at 12-hour time intervals followed by staining using Mab 4D1 as described above. It was observed that 23.8% of the infected cells were positive at 24 hours of incubation, increasing to 61.85 % after 36 hours. The dimorphism of *P. marneffei* in THP-1 was more rapid than found in liquid culture of 1% Proteose. The faster transformation of yeast cells in mammalian host cells than in liquid cell culture may suggest it being linked to better survival and increased virulence inside human and other mammalian hosts.

## INTRODUCTION

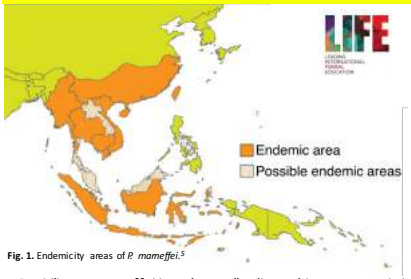



**Fig. 1.** Endemicity areas of *P. mameffei*.[5]

**Fig. 3.** *P. mameffei* in culture

**Fig. 4.** Mold-form at 25 °C

**Fig. 5.** Yeast-like form at 37°C

**Fig. 6.** Arthroconidia at 37°C

- *Penicilium marneffei* is a thermally dimorphic, opportunistic fungus endemic to Southeast Asia and parts of Asia that is responsible for the systemic human mycosis called penicilliosis.[1]
- Penicilliosis causes patients to have increase leukocyte counts, lesions on the skin and internal organs, liver enlargement, purulent pneumonia, osteolytic destruction, focal abscesses.[2]
- Infectious process occurs when conidia are inhaled from the environment (25°C) into the host's lung (37°C) where they transform into yeast cells that replicate by binary fission. and get hematogenous spread.[3]
- There are currently no commercially available serological procedures for the diagnosis of Penicilliosis marneffei, and monoclonal antibody (MAb) 4D1 is specifically reactive with *P. marneffei* yeast phase antigens.[4]
- With international travel become more widespread, together with increasing HIV infection cases in developing countries, endemic pathogenic infections like penicilliosis could post health threats to the international community.

## OBJECTIVE

This study investigated the time-dependent presence of yeast phase antigen-expressing cells in phase transition of *P. marneffei* using yeast phase specific monoclonal antibody (MAb) 4D1 in liquid cell culture (1% Proteose) and in THP-1 cell to examine the possible link of the dimorphic switching characteristic of the fungus to its virulence and survival inside the mammalian host cell.

## METHODS

### PREPARATION

**Fig. 7.** Culture and incubation of the fungus on Potato-Dextrose Agar (PDA) at 25 °C for 7 days until complete conidiogenesis. Sample was isolated from the bone marrow of an HIV infected patient diagnosed with penicilliosis.

### LIQUID CULTURE

**Liquid Culture Media Selection:**

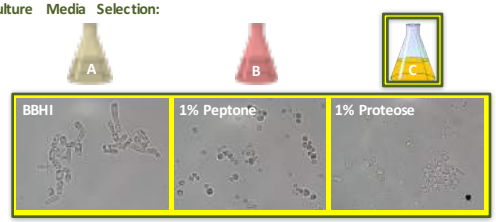



**Fig 7.** Selection of a appropriate culture media that promotes the growth of *P. marneffei* and transition to its yeast-phase form that is replicating by binary fission at 37°C. **A** arthroconidia (Bacto-Brain Heart Infusion (BBHI) by BD Biosciences); **B** minimal conidia growth (1% Bacto-Peptone by BD Biosciences); **C** yeast phase form (1% Proteose Peptone by BD Biosciences).

**Growth of *P. marneffei* in Liquid Culture:**

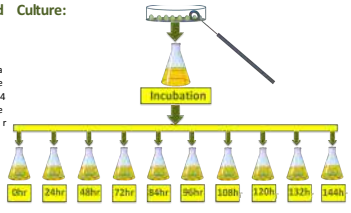



**Fig 9.** Growth of *P. mameffei* conidia (green) grown in liquid culture (inoculated at $2x10^6$ cells/ml). 24-74 hours with 24-hour collection time intervals; and 74-144 hours with 12-hour collection interval at 37°C.

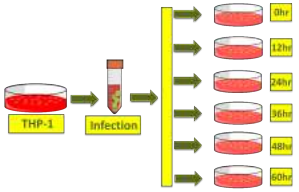
### INFECTION

**THP-1 Cells Differentiation:**


**Fig 10.** Differentiation of THP-1 monocyte cells to THP-1 macrophages (red) using 100 ng/ml phorbol myristate acetate (PMA) suspended in cell propagation media (RPMI/10% Fetal Bovine Serum (FBS)).

**Infection of THP-1 Cells:**

**Fig 11.** Infection of *P. mameffei* in THP-1 cell macrophages via phagocytosis (0-60 hours with 12 hour collection time intervals; incubated with 5% $CO_2$ at 37°C; MOI=2). Conidia that were not phagocytized were treated with 50µg/ml of nystatin to avoid extracellular conidia growth with hyphae.




### ANALYSIS

**Immunofluorescence:**

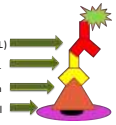
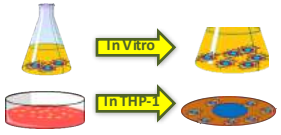



**Fig 12.** Qualitative immunofluorescence analysis of yeast-antigen expression in liquid culture (1% Proteose), and in THP-1 cell permeabilized using 0.2% Triton-X 100 in PBS.

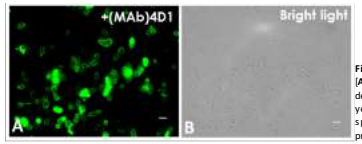
**Flow Cytometry:**

**Fig 13.** Quantitative immunofluorescence analysis by flow cytometry (AlexaFlour 488 emission wavelength gated at 519 nm) of yeast-antigen expression in liquid culture (1% Proteose Peptone) and in THP-1 permeabilized cell substrate using 0.2% Triton-X 100 in PBS.




## RESULTS

### QUALITATIVE




In Vitro

**Fig.13** Corresponding immunofluorescence [**A**] and bright field [**B**] microscopy images demonstrating the selective labeling of yeast phase *P. mameffei* by the yeast-specific MAb 4D1 in liquid culture, 1% proteose.

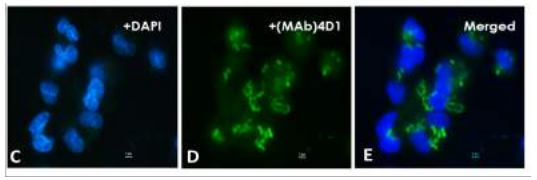



**Fig. 14** Corresponding DAPI [**C**], immunofluorescence [**D**], and merged [**E**] microscopy images demonstrating the labeling of yeast phase (**D, E**) *P. mameffei* by the yeast phase specific MAb 4D1 in THP-1 macrophage cell substrate.

In THP-1

### QUANTITATIVE

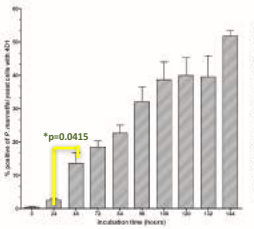



**Fig. 15** Percentage of *P. mameffei* cells grown in liquid culture positive for expression of yeast phase antigen as shown by reactivity with MAb 4D1

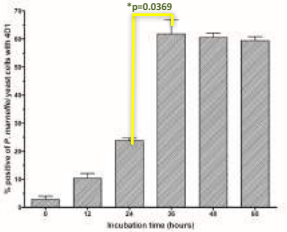



**Fig. 16** Percentage of THP-1 cells co-cultured with *P. mameffei* yeast cells positive for expression of yeast phase antigens as determined by binding to Mab 4D1.

## CONCLUSIONS

- The morphological transformation of *P. marneffei* in THP-1 cell substrate appeared to be faster than found in liquid cell culture.
- The faster transformation of yeast cells in mammalian host cells than in liquid cell culture may suggest longer survival and virulence inside human and other mammalian hosts.
- Discovery of the link between the virulence and time-dependent transition phase of *P. marneffei* may help us develop therapeutic interventions to treat penicilliosis.
- Studies on the time interval between 24-36 hours for both liquid cell culture and in THP-1 cell cultures are subjects of future work.

## ACKNOWLEDGEMENTS

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