RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2006.32.385

ScienceAsia 32 (2006): **385-393**

In vitro Growth Characterization of *Penicillium marneffei* Morphotypic Conversion

Songsak Tongchusak[1], Supinya Pongsunk[1], Pramuan Watkins[1] and Sansanee C. Chaiyaroj[1,2*]

[1] Department of Microbiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.
[2] Laboratory of Pharmacology, Chulabhorn Research Institute, Vibhavadee-Rangsit Highway, Bangkok 10210, Thailand.

* Corresponding author, E-mail: scscy@mahidol.ac.th

Received 21 Dec 2005
Accepted 29 May 2006

ABSTRACT: *Penicillium marneffei* is a dimorphic pathogenic fungus responsible for opportunistic infection in HIV infected individuals. The fungus displays dimorphic switching in response to temperature changes. At 25°C, it grows in mold form that produces penicilli conidia which are thought to be infectious. At 37°C, the fungus transforms into a fission yeast. To investigate this morphological conversion, *P. marneffei* conidia were grown in media supplemented with various organic substances. Sabouraud dextrose broth (SDB) supplemented with 4% yeast extract enhanced regular fission yeast production, whereas SDB alone and SDB supplemented with fetal bovine serum did not produce homogeneous cultures. The fungus in Sabouraud maltose broth exhibited filamentous hyphae, while its growth in Sabouraud galactose broth was inhibited. Yeast nitrogen base (YNB) medium supplemented with glucose gave hyphal growth and supplemented with N-acetylglucosamine (GlcNAc) gave retarded growth with multiseptate hyphae. Conidia-yeast conversion was not observed in YNB supplemented with glucose plus various amino acids. However, when 1% peptone was added to these YNB media, however, yeast cells or short multi-septate hyphae were produced. Surprisingly, conidia cultivated in distilled water containing 1% peptone gave uniform fission yeast similar to yeast cells found in clinical specimens. Thus, 1% peptone supplementation was responsible for the yeast transition and will serve as simple method to obtain pure *P. marneffei* yeasts for future studies on virulence, invasiveness, and pathogenesis.

KEYWORDS: *Penicillium marneffei*, dimorphic fungi, conidia-yeast conversion, arthroconidiation.

INTRODUCTION

Penicillium marneffei is a newly emerging opportunistic dimorphic fungal pathogen causing a systemic disease in immunocompromised individuals, especially HIV infected patients[1,2]. Cell type switching in dimorphic fungi is thought to be modulated by environmental factors via cytoplasmic signal transduction. The fungus undergoes morphological switching mainly in response to temperature changes. At normal ambient temperature (25°C), *P. marneffei* grows in mold form, producing large amounts of long chain penicilli conidia. However, under a stress temperature (37°C) the fungus undergoes arthroconidiation and develops into fission yeast[3]. The yeast form of *P. marneffei* is of pathological relevance and resides intracellularly during host infection. It has been proposed that *P. marnefei* infection is initiated by inhalation of its airborne conidia. The conidia are then ingested by alveolar macrophages in lung tissue where they germinate and convert to fission yeast cells inside the macrophages. These antigen presenting cells then serve as delivery vehicles that spread the fungus to other parts of the body[4].

Brain heart infusion (BHI) broth at 37°C has commonly been used to initiate the morphological transition of *P. marneffei* from hyphae to yeasts. *In vitro* yeast transition in this particular medium always results in a mixed population of yeast cells and short hyphae that do not resemble the yeast cells found in clinical specimens. Therefore growth conditions suitable for cultivation of yeasts resembling those from penicilliosis patients were explored since the discovery of a simple method for *P. marneffei* dimorphic switching would be useful for future investigations on yeast-host cell interactions and the characterization of *P. marneffei* genes regulating dimorphism.

MATERIALS AND METHODS

Fungal Strains

Penicillium marneffei strains used in this study were reference strain (ATCC 64102) purchased from the American Type Culture Collection, MD, USA, and 8

local strains, namely RT-45, RT-54, RT-58, RT-72, AR-5, AR-70, MK-76, and BE-63. The local strains were isolated from penicilliosis patients from different parts of Thailand. The organisms were maintained as mycelial (mold) phase on SDA at 25°C under aerobic conditions.

Preparation of Inoculum

Sterile 0.01% Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) in distilled water was added to 1 to 2 week-old *P. marneffei* slant cultures. The conidial suspension was collected and conidial numbers were determined using a hemacytometer (Bright-Line® Improved Newbauer Hemacytometer).

Culture Media

Complex media used in this study were BHI broth (Difco, Becton Dickinson, MD, USA) supplemented with or without 10% fetal bovine serum (FBS) supplement (Gibco BRL, Grand Island, NY, USA), Sabouraud dextrose broth (SDB) (2% peptone and 4% glucose), SDB with 10% or 20% FBS, SDB with 4% yeast extract (YE) (Difco), and SDB with 4% YE plus 10% FBS. Two additional SDB based media were supplemented with 4% galactose (Difco) (SGB) or 4% maltose (Difco) (SMB) instead of 4% glucose (Difco). All cultures were observed for 13 days.

To study the effect of various concentrations of glucose and amino sugars on yeast transition, a defined medium with bacto yeast nitrogen base without amino acids (YNB) (Difco) was used. YNB, the basal medium, consists of 0.5% ammonium sulfate, vitamins, trace elements, and salts that are generally essential for yeast and hyphal growth. The effect of glucose concentration on yeast transition of *P. marneffei* was determined by using YNB supplemented with 0.1%, 0.5%, 1%, 2%, or 4% glucose. YNB supplemented with 1 mM, 2.5 mM, 5 mM, or 10 mM N-acetyl-D-glucosamine (GlcNAc) (Sigma-Aldrich) was used in order to verify the importance of GlcNAc in yeast transition. Influence of specific nitrogen sources on *P. marneffei* yeast-transition was also studied by growing fungal conidia in YNB supplemented with 0.5% glucose and each of the following 11 amino acids: L-arginine, L-cysteine, L-glutamic acid, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline and L-tryptophan. Most amino acids were purchased from Sigma-Aldrich (St. Louis, MO, USA) except for L-leucine and L-phenylalanine that were purchased from Merck (Darmstadt, Germany). The final concentration of each supplemented amino acid was 100 mg/L. The effect of peptone (Difco) on *P. marneffei* yeast transition was subsequently investigated. One percent peptone in distilled water and in YNB was used. Moreover, its transition ability was investigated daily when 1% peptone was added to the YNB supplemented with 0.1%, 0.5%, 1%, 2%, or 4% glucose and to the YNB supplemented with 1 mM, 2.5 mM, 5 mM, or 10 mM GlcNAc.

Determination of *P. marneffei* Conidia-to-Yeast Transition

Approximately $1x10^7$ conidia were inoculated into a 250-ml flat bottom flask containing 50 ml of the tested liquid media. The culture was incubated in a gyratory shaker (New Brunswick Scientific Co., Inc.) at 37°C, 120 rpm. One milliliter of culture broth from each condition was sampled, and prevalence of yeast morphology was assessed directly under a light microscope (Olympus BH-2, Tokyo, Japan). Nuclear staining with 500 mg/ml of 4′6′-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) and and cell wall staining with 500 mg/ml of calcofluor white (CAL) (Sigma-Aldrich) were observed using an epifluorescent microscope (NikonLOBOPHOT-2). Yeast cell numbers was determined using a hemacytometer.

Results

Effect of FBS on the Transition of *P. marneffei* Yeast in BHI Medium

In BHI medium strains ATCC64102, RT-45, RT-54 and AR-5 produced only a few yeast cells and short hyphae, whereas strains RT-58, RT-72, AR-70, MK-76 and BE-63 conidia converted to yeasts more readily. Since the recent clinical isolate, RT-72, exhibited typical dimorphic characteristics of *P. marneffei*, it was used in further experiments. No difference in the growth and morphology was observed when 1×10^7 conidia of *P. marneffei* strain RT-72 were inoculated in 50 ml of BHI broth with and without 10% FBS (Fig. 1A-D). In both cultures conidia swelled and produced germ tubes overnight. Germ tubes elongated and developed into septate hyphae. In BHI containing 10% FBS, each hyphal segment subsequently splits into arthroconidia within 4 days (Fig. 1B). However, development of arthroconidia was somewhat delayed in FBS-free BHI and high number of short hyphae could be observed (Fig. 1A). Each arthroconidium finally divided by binary fission into a unicellular yeast cells within 6 days. In comparison to BHI containing FBS, the yeast cells in unsupplemented BHI were more oval in shape (Fig. 1C). Regardless of FBS supplementation, almost all the short hyphae converted to yeast cells within 6 days (Fig.1D). After 6 days, all fungal cells started to degenerate (data not shown).

Transition Ability of *P. marneffei* in SDB, SDB Supplemented With FBS and Yeast Extract Medium

Yeast cells converted in SDB were heterogeneous in size and shape and the conidia tended to grow into

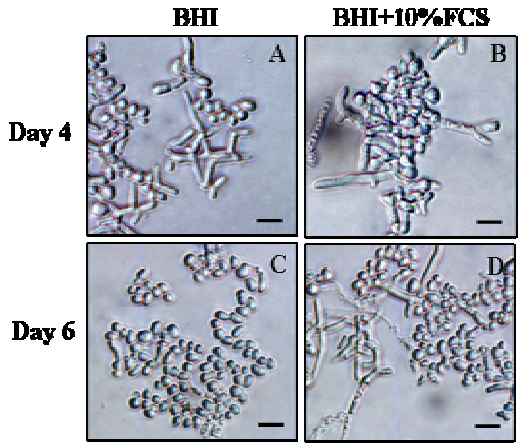

Fig 1. Morphology of *P. marneffei* strain RT-72 cultured in liquid BHI (A and C) and in liquid BHI supplemented with 10% FBS (B and D) at 37°C observed at Day 3 and Day 6. Bar length is 10 μm.

short hyphae (Fig. 2A). With addition of FBS and YE or combinations of these (Fig. 2B-2E), the fungal cells exhibited similar morphology to that cultivated in BHI (Fig. 1A). Maximal growth was obtained at Day 5 and 6. A mixture of short hyphae and yeast cells with a high density of intracellular granules was observed. Notably, yeast cells that switched under 4% YE (Fig. 2D) displayed unicellular oval and were larger in size and healthier in appearance than those cultivated in the BHI. They were also more homogeneous in size and shape.

Effect of Carbon Source on Transition

Germinated conidia did not grow in SGB medium either with or without 4% YE (Fig. 2F) while conidial conversion to small number of yeast cells was observed when grown in SMB with or without 4% YE medium (Fig. 2G). In SGB, conidia swelled and started to produce a germ tube within 24 hours. After 48 hours, the germinated conidia grew isotropically to form spherical yeasts with a deformed germ tube (Fig. 2F). Prolonged incubation of this culture led to growth inhibition (data not shown). However, the yeast cells obtained from both conditions of SMB (Fig. 2G) were less uniform in size and shape when compared to those from SDB with 4% YE (Fig. 2D).

Effect of Glucose Concentration on Transition

Germinated conidia could not grow normally in YNB lacking ammonium sulfate. However, in the presence of ammonium sulfate and glucose, conidia swelled and germinated into hyphal cells that had a thinner compartments than those obtained with BHI and SDB after 2 day's incubation. At Day 7 the hyphae began to die without any conversion to yeast cells. In YNB media with low concentrations of glucose (0.1% and 0.5%), mycelia with high frequency multiseptation were found (Fig. 2H). With high glucose concentrations, only thin hyphal cells were observed (Fig. 2I). Despite this, hyphal mass increased significantly when more glucose was added to YNB (data not shown).

Effect of Various Amino Acids on Transition

No particular amino acid added to YNB containing 0.5% glucose contributed to the transition of *P. marneffei* to yeast cells. Conidia grown in all conditions germinated to produce hyphae within 2 days. Hyphal

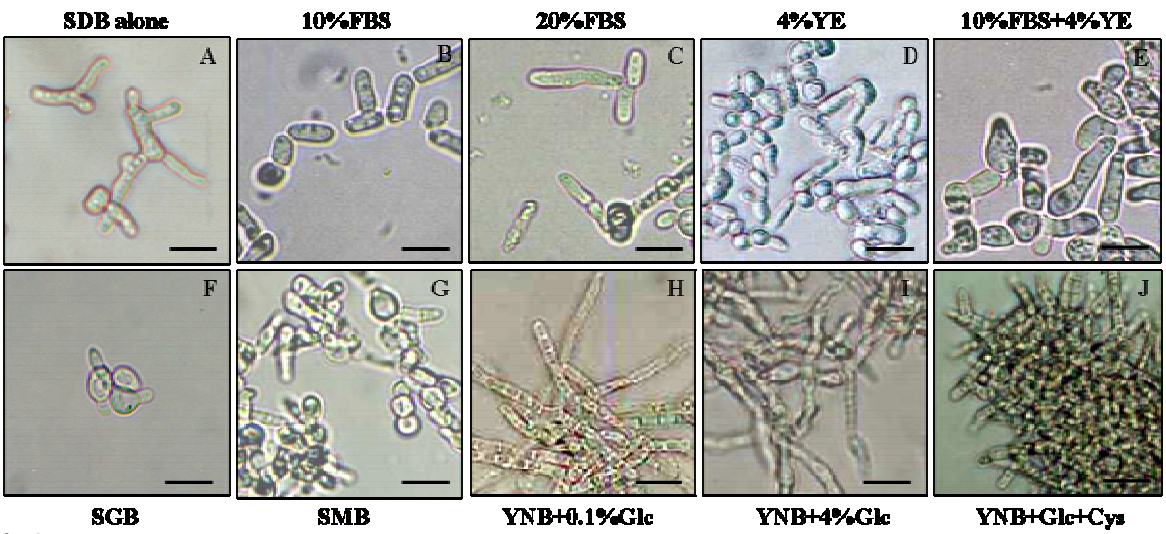

Fig 2. Day 6 morphology of *P. marneffei* strain RT-72 cultured in SDB (A), SDB with 10% FBS (B), SDB with 20% FBS (C), SDB with 4% YE (D), and SDB with 10% FBS + 4% YE (E). The cultures were maintained at 37°C. Effect of carbohydrate supplements on growth was evaluated in SGB (F), SMB (G), YNB+0.1%Glc (H), YNB+4%Glc (I), and YNB with Glc+Cys at 37°C. Bar length is 10 μm.

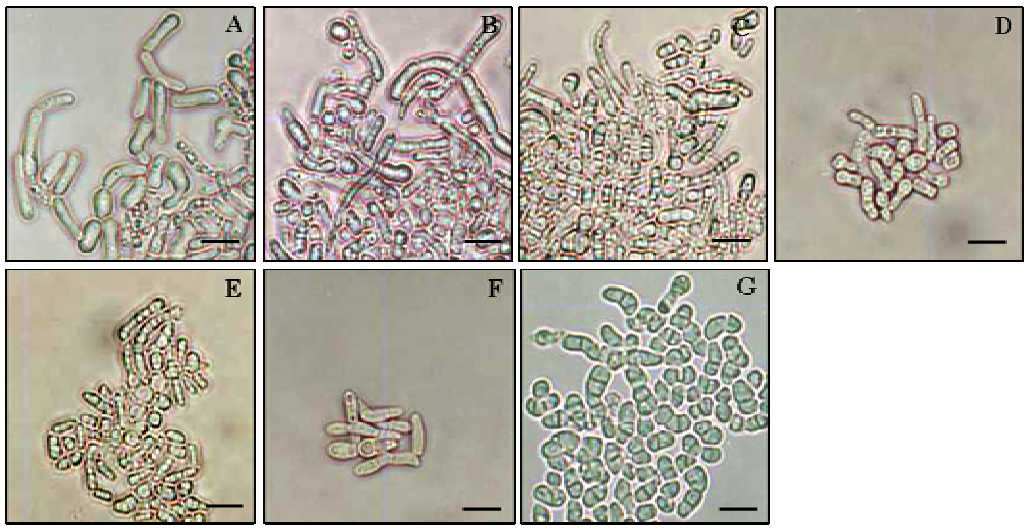

Fig 3. Morphology of *P. marneffei* strain RT-72 cultured in YNB with 4% glucose + 1% peptone (A), YNB with 2% glucose + 1% peptone (B), YNB with 1% glucose + 1% peptone (C), YNB with 0.5% glucose + 1% peptone (D), and YNB with 0.1% glucose + 1% peptone (E), YNB + 1% peptone (F), and DW + 1% peptone (G) at 37°C. Bar length is 10 μm.

cells grew apically to form elongated hyphae or small mycelia until Day 8 (Fig. 2J), and only degenerated hyphal cells were detected thereafter.

Effect of Peptone on Transition

Since no single amino acid was found to be favorable to conidia-yeast transition, the effect of peptone, a complex organic nitrogen source, on the transition was further explored. As with SDB, conidia cultured in YNB with 4% glucose + 1% peptone (Fig. 3A) and YNB with 2% glucose + 1% peptone (Fig. 3B) grew apically to form hyphae and then the hyphal cells divided into arthroconidia. High numbers of short hyphae were observed on Day 8 for YNB with 4% glucose + 1% peptone and on Day 10 for YNB with 2% glucose + 1% peptone. A mixed population of yeast cells, short hyphae and several dead cells were observed.

Germinated conidia were detected at 3 days in YNB with 1% glucose + 1% peptone (Fig. 3C), in YNB with 0.5% glucose + 1% peptone (Fig. 3D), in YNB with 0.1% glucose + 1% peptone (Fig. 3E). Short hyphal cells with multiple septa were found within 10 days. Yeast cells were significantly smaller in size than those produced at higher glucose concentrations [i.e., in YNB with 4% glucose + 1% peptone (Fig. 3A), or in YNB with 2% glucose + 1% peptone (Fig. 3B)].

When conidia were inoculated in YNB + 1% peptone or in 1% peptone in distilled water (Fig. 3F and 3G), yeast cells similar to those found in clinical specimens were produced. Most germinated conidia did not require an elongation process to form hyphae, and arthroconidia were not observed. Instead, conidia grew and divided by septation to form fission yeasts. High concentrations of glucose gave the opposite effect. Hyphae formed in media with 4% and 2% of glucose despite the presence of 1% peptone (Fig. 3A and 3B). Strikingly, yeasts grown in distilled water supplemented with 1% peptone gave a morphology similar to that found in human immune cells.

Effect of N-Acetyl-D-Glucosamine on Transition

RT-72 in YNB medium containing ammonium sulfate as a nitrogen source, supplemented with various concentrations of GlcNAc (1 mM, 2.5 mM, 5 mM, and 10 mM) as the sole carbon source gave hyphal cells with many vacuoles (Fig. 4A-D). Short hyphae with multiple septa were observed in all tested conditions and dead cells were seen within 8 days. The morphology of yeast cells did not resemble that of cells in BHI or in clinical specimens. In order to investigate the effect on conidia-yeast transition, 1% peptone was added to the YNB-GlcNAc medium. The following media were tested: YNB with 1 mM GlcNAc + 1% peptone (Fig. 4E); YNB with 2.5 mM GlcNAc + 1% peptone (Fig. 4F); YNB with 5 mM GlcNAc + 1% peptone (Fig. 4G); YNB with 10 mM GlcNAc + 1% peptone (Fig. 4H). When compared to peptone-free controls, 1% peptone added to culture gave a significant slower growth.

Fungal Strains Comparison

Growth of *P. marneffei* strain RT-72 on BHI, SDB with 4% yeast extract, and 1% peptone was compared. Conidia grown in BHI and SDB with 4%YE began to swell and develop into germ tubes after overnight culture. Then the germ tubes elongated to form mycelia within 3-4 days and underwent arthroconidiation

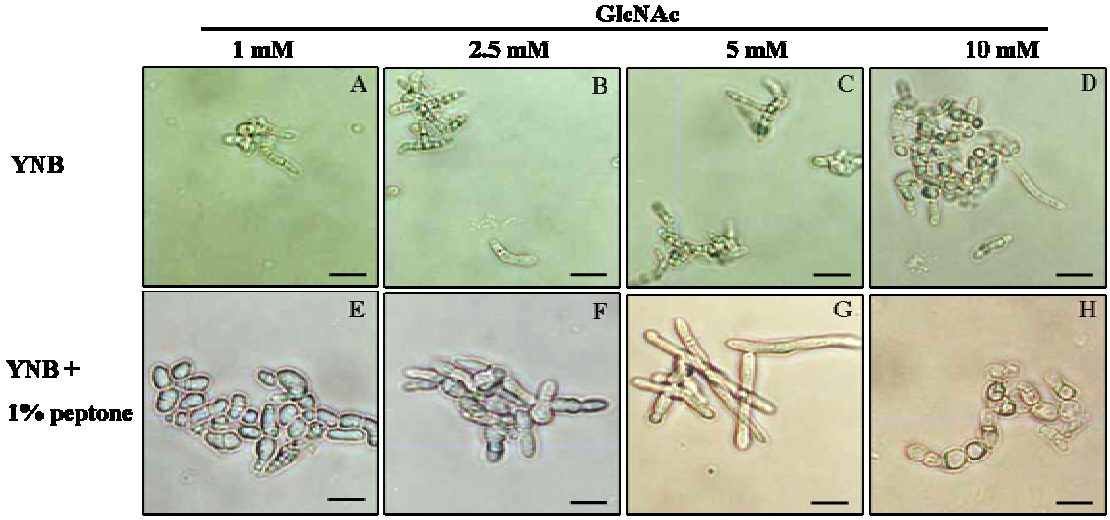

Fig 4. Morphology of *P. marneffei* strain RT-72 cultured in YNB with 1 mM GlcNAc (A), YNB with 2 mM GlcNAc (B), YNB with 5 mM GlcNAc (C), and YNB with 10 mM GlcNAc (D) at 37°C for 6 days and YNB with 1 mM GlcNAc + 1% peptone (E), YNB with 2.5 mM GlcNAc + 1% peptone (F), YNB with 5 mM GlcNAc + 1% peptone (G), and YNB with 10 mM GlcNAc + 1% peptone (H) at 37°C for 14 days. Bar length is 10 μm.

within 5 days (Fig. 5). By Day 5 a mixture of arthroconidia and some short hyphae was seen. The growing arthroconidia then divided by septum formation in the middle of cells leading to generation of fission yeast cells. A homogeneous culture of yeast cells in these three media was obtained within 6-7 days. A number of yeast cells exhibited cytoplasmic vacuoles and started to die after Day 8. The sizes of yeast cells in both media were approximately 5-10 x 8-20 μm. In 1% peptone at 37°C, some conidia swelled and germinated within a day. On Day 2 the germinated conidia grew, and a septum was evident in the middle of cells. Hyphal cells

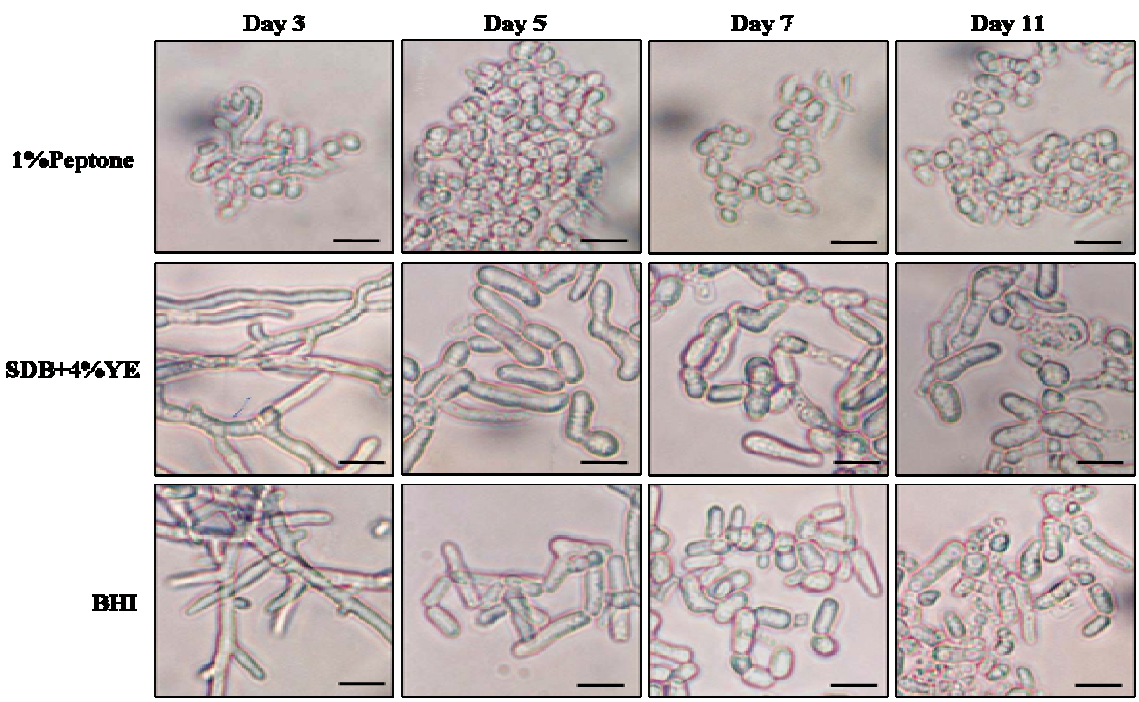

Fig 5. Morphology of *P. marneffei* yeasts strain RT-72 grown in 1% peptone, SDB + 4% YE and BHI observed under bright field at Days 3, 5, 7 and 11. Bar length is 10 μm.

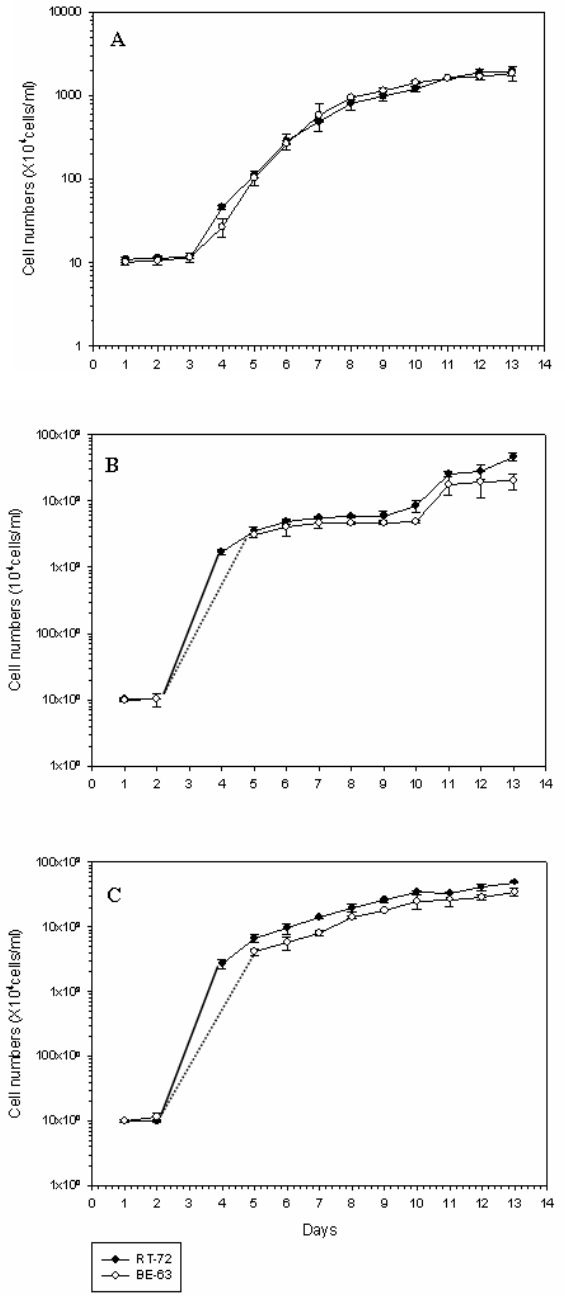

Fig 6. Growth curve of *P. marneffei* strains RT-72 and BE-63 grown in 1% peptone (A), SDB + 4% YE (B), and BHI (C) observed within the period of 13 days. Closed square represents RT-72; open square represents BE-63.

were not found (Fig. 5). The conidia did not grow at the apical end to form hyphae but swelled and directly divided by binary fission to form fission yeasts. The size of yeast cells generated in this medium was 5-7 x 5-8 μm (Fig. 5).

The growth of tested strains were measured by counting the number of yeast cells daily from Day 1 to Day 13 in 3 independent experiments to produce mean yeast cell numbers to plot growth curves (Fig. 6A-C). Upon addition of *P. marneffei* strains RT-72, RT-58, AR-70, MK-76 and BE-63 at a concentration of 1 x 10^5 conidia/ml into the 1% peptone medium, some conidia grew and cell septa were detected within a day. The number of yeast cells gradually increased. At Day 8 approximately 3 x 10^6 yeast cells/ml were found in the cultures of *P. marneffei* strains MK-76, RT-58 and AR-70 (data not shown). The numbers of yeast cells were approximately 30-fold higher in the cultures of RT-72 and BE-63 than the other strains (Fig. 6A). *P. marneffei* strains grown in 1% peptone showed a very slow doubling time. After this period (Day 8-Day 13), although newly formed yeast cells could still be detected, some cells began to die and this resulted in stable yeast cell numbers.

With conidia of *P. marneffei* strains RT-72, RT-58, AR-70, MK-76 and BE-63 grown in BHI or SDB with 4% YE, yeast cells were not observed until Day 4. In BHI, yeast cells of *P. marneffei* strain RT-72 reached approximately 5.6x10^7 cells/ml and those of BE-63 reached approximately 4.9x10^7 cells/ml by Day 8 (Fig. 6C). After Day 8, the number of RT-72 yeast cells remained constant until Day 13. In SDB with 4% YE, approximately 1.96x10^8 cells/ml were obtained with RT-72 and 1.39x10^8 cells/ml with BE-63 by Day 7 (Fig. 6B).

Yeast Morphology and Cell Wall Integrity

Most yeast induced by 1% peptone and stained with DAPI exhibited a single nucleus per cell (Fig. 7). Yeast cells cultured in BHI or SDB with 4% YE also had a single nucleus per cell, although more than one nucleus was found in short hyphae (Fig. 7). When lyticase and chitinase digestions were performed, approximately 70% of the digested yeast cells from SDB with 4% YE medium became protoplasts, while only 10% of those from cells 1% peptone culture did (data not shown). The results demonstrate that the cell wall of yeast cells from 1% peptone culture are more tolerant to lyticase and chitinase digestions than yeast cells from SDB + 4% YE, perhaps due to the differences in their cell wall compositions.

Discussion

In vitro studies by Cogliati *et al* in 1997 showed that *P. marneffei* conidia phagocytized by the J774 murine macrophage-like cell line, replicated by fission within 24 hours to form yeast cells[4], hence the relevance in employing *P. marneffei* yeasts to study the pathogenesis of *P. marneffei* infection. Previously, BHI medium was commonly used to generate *P. marneffei* yeast cells[6, 7]. These cells develop via arthroconidiation, a process whereby conidia differentiate to hyphal cells that subsequently divide into arthroconidia by septal

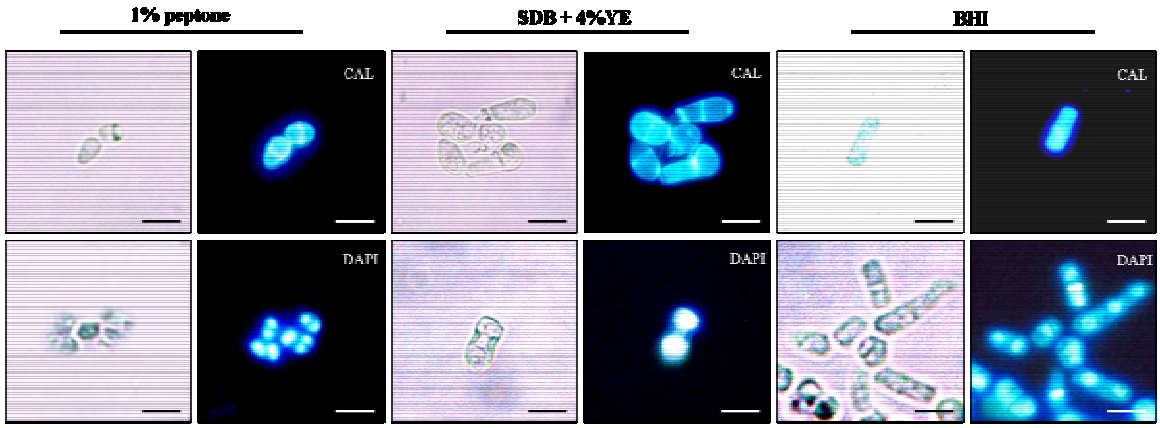

Fig 7. Cell wall, septum and nuclear morphology of *P. marneffei* yeasts strain RT-72 grown in 1% peptone, SDB + 4% YE and BHI. The left panels show fungal cells observed under bright field microscope. The right panels illustrate cells stained with calcofluor and DAPI observed under epifluorescence. Bar length is 10 μm.

formation. Each arthroconidium further divides by fission to form yeast cells. This occurs in BHI medium during the late stage of growth (Fig. 6). Thus, BHI cultures always contain a mixture of yeast cells and arthroconidia mixed with dead cells.

By contrast, we were able to produce a homogeneous population of fission yeasts within 24 hours using 1% peptone broth at 37°C. The size and shape of the yeast cells obtained from this medium were similar to those found in clinical specimens (Fig. 8). Consequently, 1% peptone solution seems to be the medium-of-choice for induction and maintenance of *P. marneffei* in the yeast form. To date, establishment of a hyphal network by *P. marneffei* conidia has not been detected in macrophages[1,2]. As a result, Andrianopoulos suggested that a direct conidium-to-yeast cell morphogenesis program might be activated by certain factors within macrophages[3]. In this study, however, we have demonstrated direct conversion from conidia to yeast outside host cells using 1% peptone medium. Hence, an additional path in the life cycle of *P. marneffei* exists from conidia-to-yeast cell by binary fission, bypassing the process of arthroconidiation.

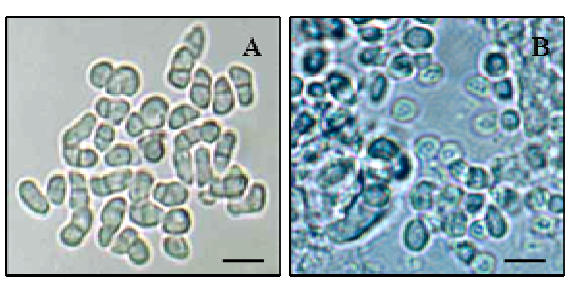

Fig 8. Comparison of *P. marneffei* yeast-like cells from *in vitro* induction, 1% peptone (A) and clinical specimen, oral tissue section from a patient (B). Bar length is 10 μm.

In an attempt to gain more information on the effects of serum on the yeast transition, phase conversion in BHI with or without FBS was investigated. We found that serum had no effect on the conidia-to-yeast transition in *P. marneffei*. This contrasts with results of previous studies on the effects of serum on morphogenetic changes in other fungi[8, 9]. Serum is reported to be the most powerful inducer for germ-tube formation in *C. albicans*[8], which normally resides in oral or vaginal cavities with little serum exposure. Feng *et al.* further reported that a fraction of serum filtrate lower than 1 kDa in size contained active ingredients for germ-tube formation in *C. albicans*[9]. Conversion from yeast to germ-tube upon serum exposure was proposed as a mechanism that allows the organism to evade or divert host immune response. Indeed, Vazques-Torres and Balish showed that normal phagocytic cells engulfed germ-tube cells with much less efficiency than yeast cells[10]. Moreover, neoantigens expressed on the surface of germ tube cells did not contain any of the dominant and protective immunodominant epitopes that are normally expressed on the yeast cell surface[11]. For *S. cerevisiae*, factors such as glucose and other carbohydrates, nitrogen sources, and short-chain alcohols are also involved in its filamentous growth[12, 13]. The fact that the addition of serum did not affect *P. marneffei* conidium-to-yeast transition suggests that it is mediated by factors that are distinct from those for *C. albicans* and *S. cerevisiae*. The difference in the sites of infection of these fungi in relation to their serum exposure should also be taken into consideration.

Our tests on the influence of glucose on conidium-to-yeast transition revealed that glucose addition to 1% peptone medium inhibited the transition. The higher the concentration of glucose added to the media, the

more hyphal mass was generated. This contrasts with result of previous studies on the effects of glucose on fungal morphogenesis. In *M. rouxii* glucose inhibits the initiation of filamentation[14]. Similarly, it inhibits germ-tube formation in *C. albicans*[15]. Pollack and Hashimoto[15] further suggested that glucose might generate catabolite intermediates that interfere with hyphal morphogenesis in *C. albicans*. In *P. marneffei*, however, glucose and other sugars have a negative effect on the conidia-yeast transition. Moreover, *P. marneffei* conidia displayed no growth in yeast nitrogen base medium supplemented with galactose. The fact that galactose did not support the growth of *P. marneffei* in this study was in agreement with a previous report by Wong *et al*[16] on the inhibition of *P. marneffei* growth by galactose.

The ability of the medium containing only 1% peptone (as a sole carbon and nitrogen source) to support growth of *P. marneffei* is not surprising. An extensive study in *A. nidulans* and *N. crassa* by Kinghorn and Pateman[17] found that both fungi can use amino acids as the sole nitrogen and carbon source, albeit inefficiently in most cases. In general, amino acids that can degrade to intermediates of carbon metabolism by one or two reactions are better sources than those that require more degradation steps. However, it is important to be aware that when an amino acid is utilized as a sole source of carbon, production of a large amount of ammonia occurs as a consequence. Since the amount of reduced carbon required for respiration is far higher than the nitrogen requirement for growth, ammonia accumulates excessively, leading to growth inhibition as a result of ammonia toxicity or high pH levels[18]. This might explain the results of our study, in which low doubling time as well as low growth rate were observed for yeast cell growth in 1% peptone.

In summary, the results from this study demonstrate that it is possible to induce *in vitro* conversion of *P. marneffei* from conidia to yeast cells. Nevertheless, since medically important fungi carry an impressive array of macromolecules with immunomodulatory functions, more biochemical, immunological, and molecular characterization is necessary in order to compare these yeast cells to those that reside in macrophages. More precisely, cell wall and antigenic compositions should be determined to confirm that the yeast cells generated from this medium can be used as a representative for the pathogenic yeasts in penicilliosis patients. This is critical for studies on interaction with host immune cells in *vitro*. An understanding of how a normal host resists fungal invasion will demonstrate how specific immune defects, present in AIDS patients, are fundamental for the development of rational approaches to immunodiagnosis and therapy of *P. marneffei* infections.

Acknowledgements

This work was supported by National Science and Technology Development Agency (NSTDA). S.T. and S.P. are Graduate Students of the Royal Golden Jubilee (RGJ) Program, the Thailand Research Fund (TRF). We thank, Dr. Candace Rudmose and Praveen Chansrichavala for critical review of the manuscript.

References

1. Garrison RG and Boyd KS (1973) Dimorphism of *Penicillium marneffei* as observed by electron microscopy. *Can J Microbiol* **19**, 1305-9.
2. Chan YF and Chow TC (1990) Ultrastructural observations on *Penicillium marneffei* in natural human infection. *Ultrastruct Pathol* **14**, 439-52.
3. Andrianopoulos A (2002) Control of morphogenesis in the human fungal pathogen *Penicillium marneffei*. *Int J Med Microbiol* **292**, 1-18.
4. Cogliati M, Roverselli A, Boelaert JR, Taramelli D, Lombardi L and Viviani MA (1997) Development of an *in vitro* macrophage system to assess *Penicillium marneffei* growth and susceptibility to nitric oxide. *Infect Immun* **65,** 279-84.
5. Simonetti N Strippoli V and Cassone A (1974) Yeast-mycelial conversion induced by N-acetyl- D-glucosamine in *Candida albicans*. *Nature* **250**, 344-6.
6. Jeavons L, Hamilton AJ, Vanittanakom N, Ungpakorn R, Evans EGV, Sirisanthana T and Hay RJ (1998) Identification and purification of specific *Penicillium marneffei* antigens and their recognition by human immune sera. *J Clin Microbiol* **36**, 949-54.
7. Kudeken N, Kawakami K and Saito A (1998) Different susceptibility of yeasts and conidia of *Penicillium marneffei* to nitric oxide (NO)-mediated fungicidal activity of murine macrophage. *Clin Exp Immunol* **112**, 287-93.
8. Brown AJ (2002) Morphogenetic signaling pathways. In: *Candida albicans.* In: *Candida and Candidiasis, 1st ed* (Edited by Calderone RA), pp95-106. ASM Press, Washington DC.
9. Feng Q, Summers E, Guo B and Fink GR (1999) Ras signaling is required for serum induced hypha l differentiation in *Candida albicans. J Bacteriol* **181**, 6339-46.
10. Vazques-Torres A and Balish E (1997) Macrophages in resistance to candidiasis. *Microbiol Mol Biol Rev* **61**, 170-92.
11. De Bernardis F, Molinari A, Boccanera M, Stringaro A, Robert R, Senet JM, Arancia G and Cassone A (1993) Modulation of cell surface-associated mannoprotein antigen expression in experimental *Candida* vaginitis. *Infect Immun* **62**, 509-19.
12. Lorenz MC, Pan X, Harashima T, Cardenas ME, Xue Y, Hirsch JP and Heitman J (2000) The G protein-coupled receptor GPR1 is a nutrient sensor that regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Genetics* **154**, 609-22.
13. Lorenz MC, Cutler NS and Heitman J (2000) Characterization of alcohol-induced filamentous growth in *Saccharomyces cerevisiae*. *Mol Biol Cell* **11**, 183-99.
14. Bartniki-Garcia S (1968) Control of dimorphism in *Mucor* by hexoses inhibition of hyphal morphogenesis. *J Bacteriol* **96**, 1586-94.
15. Pollack JH and Hashimoto T (1987) The role of glucose in the pH regulation of germ-tube formation in *Candida albicans*. *J Gen Microbiol* **133**, 415-24.

16. Wong S, HO T, Ngan A, Woo P, Que TL and Yuen KY (2001) Biotyping of *Penicillium marneffei* reveals concentration dependent growth inhibition by galactose. *J Clin Microbiol* **39**, 1416-21.
17. Kinghorn JR and Pateman JA (1977) Nitrogen metabolism. In: *Genetics and Physiology of Aspergillus*, 1st ed (Edited by Smith JE and Pateman JA), pp147-202. Academic Press, New York.
18. Pateman JA and Kinghorn JR (1976) Nitrogen metabolism. In: *The Filamentous Fungi Volume 2 Biosynthesis and Metabolism*, 1st ed (Edited by Smith JE Berry DR), pp 159-223.: Edward Arnold, London.