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| **Thesis Title** | Immunological Studies of *Penicillium marneffei* Antigens |
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## ABSTRACT

Penicilliosis marneffei is a disseminated and progressive infection caused by *Penicillium marneffei*, the only dimorphic species of the genus. In the past, the disease has been recognized infrequently. More recently, the spread of AIDS epidemic has led to such a sharp increase in the incidence of penicilliosis marneffei that it has emerged as the third most common infectious complication in AIDS patients residing in northern Thailand. Many cases have been misdiagnosed as other invasive diseases such as tuberculosis, pneumocystis carinii pneumonia, histoplasmosis, and other mycoses due to their similar clinical manifestations and histopathological responses. Incorrect diagnosis and delayed treatment have contributed to a high mortality rate especially in those immunocompromised hosts. Moreover, lifelong therapy should be maintained to prevent relapses. At present, very limited information regarding mycological and immunological aspects of penicilliosis marneffei is available.

In this study, an attempt was made to develop appropriate cultural conditions for *in vitro* conversion of mycelial to yeast form. *P. marneffei* mycelial form has undergone a transitional stage to yeast form upon repeated subculturing on brain heart infusion agar supplemented with 10% fetal calf serum (BHIS) at 37 °C. A variety of antigenic extracts from mycelial and yeast cultures were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Concanavalin A stained SDS-PAGE demonstrated the strongest banding patterns compared to those stained with silver and Coomassie brilliant blue.

Immunoblots of different extracts were performed against human serum from penicillosis marneffei patient, rabbit sera raised against culture filtrate extracts of *P. marneffei* mycelial form and rabbit sera raised against culture filtrate extracts of *P. marneffei* yeast form. An antigen with molecular weight of 38 kD specific to *P. marneffei* was identified. This immunoreactive antigen was present exclusively in all *P. marneffei* extracts although in varying degrees. It was absent in culture filtrate extracts of *Histoplasma capsulatum*, *Aspergillus fumigatus*, *A. niger*, *Penicillium* species other than *marneffei*, *Candida albicans* and *Cryptococcus neoformans.* The biological and chemical characteristics of this 38-kD component remain to be elucidated.

Further analysis was taken to evaluate the potential of this immunoreactive 38-kD *P. marneffei* antigen as a possible candidate antigen in diagnosing penicilliosis marneffei. 579 sera from HIV-seropositive patients and 313 sera from HIV-seronegative individuals were screened for 38-kD antibodies using immunoblot analysis. The 38-kD *P. marneffei* specific antigen has been proven of diagnostic value. Approximately 50% of sera obtained from culture-confirmed penicilliosis marneffei patients presented strong antibody responses to this antigen whereas less than 1% of sera from HIV-seronegative individuals gave a positive reaction. In addition, the presence of antibody to this immunoreactive antigen in 17% of serum samples from asymptomatic HIV persons residing in the endemic area might be an evidence of subclinical infection in them. The fact that coexistence of opportunistic

infections is frequently found among immunocompromised hosts was supported by the positive reactivity of serum specimens obtained from those HIV-seropositive patients with other systemic mycoses (28% for cryptococcosis and 21% for candidiasis) to the 38-kD antigen. These observations may be useful for future development of diagnostic serologic assay for *P. marneffei* infection.