I. INTRODUCTION

Penicillium marneffei is a dimorphic pathogenic fungus endemic in Southeast Asia and Southern parts of China. The mycelial phase grows best at 25°C -27°C and the yeast phase grows best at 37°C (as might be expected). The yeast phase is the pathogenic form found in human tissues. Prior to AIDS, infection with *P. marneffei* was a rare event. The organism was first isolated from bamboo rats, *Rhizomys sinensis*, in Vietnam (Capponi et al, 1956) and subsequently from another species of bamboo rat, *R. pruinosus*, in southern China (Deng et al, 1986). These animals are believed to be an important reservoir of infection since most are chronically infected with the fungus.

In Thailand, the organism was isolated from *Rhizomys pruinosus, Cannomys badius* (Ajello et al, 1995) and from *R. sumatrensis* and the reddish-brown *C. badius* (Chariyalertsak et al, 1996 b). However, the actual route of human infection with this organism is not known. Rats may be the source of human infection, but it is also conceivable that rats are just another incidental host. The organism may be like the other endemic fungal pathogens in that it is predominantly a soil fungus, and then the human and animals acquired its conidia by inhalation. The fungus is endemic in Southeast Asia, especially Vietnam, Thailand (particularly the northern part around Chiang Mai), and the southern part of China (notably the Guangxi province, west of Guangzhou). Consequently, infection has been seen predominantly in residents of these areas. In the West, penicilliosis is seen in HIV-positive immigrants from the endemic area as well as persons who traveled there.

Penicilliosis has become a very common infection in the endemic area. In the northern Thailand, Chiang Mai province, infection with *P. marneffei* is the second or third most common opportunistic infection occurring after tuberculosis and cryptococcosis and occurs in up to one third of HIV-infected patients. The organism proliferates in macrophages and is disseminated throughout the body, especially to the reticuloendothelial system. Consequently, liver, lymph nodes, bone marrow and spleen are commonly involved. Common manifestations of disseminated *P. marneffei* infection in AIDS patients were fever, anemia, weight loss, lymphadenopathy,

hepatosplenomegaly, respiratory signs and skin lesions (Chiewchanvit et al, 1991; Supparatpinyo et al,1994). The severity of the disease depended on the immunological status of the individual. Most symptoms of penicilliosis marneffei unspecify and mimic acute histoplasmosis, chronic tuberculosis and other systemic infections. Thus, the correctly and rapidly diagnosis are important. Nearly all of patients who were diagnosed responded initially to amphotericin B or itraconazole, whereas, most who were not diagnosed and treated died (Supparatpinyo et al, 1994).

Presumptive diagnosis of *P. marneffei* can be made by microscopic examination of Wright's stained bone marrow aspirates and/or touch smear of skin biopsy or lymph node biopsy specimens (Supparatpinyo et al, 1992). The organism appears as unicellular round to oval or elongated cells which may divide by cross-wall formation in macrophages or histiocytes. The diagnosis has to be confirmed by direct culture of the organism. The typical features of this fungus is its thermal dimorphism (Segretain,1959). It grows as a mold with a characteristic of the genus *Penicillium* on artificial media at 25°C, and as a yeast form at 37°C. These conventional means are always time consuming. Therefore, serodiagnosis of *P. marneffei* infection needs to be improved, based on diagnostic tests which would enable the identification of either those individuals with initial asymptomatic forms of disease or those demonstrating nonspecific symptoms of *P. marneffei* infection. The exoantigen test for *P. marneffei* was the first established by Sekhon et al (1982, 1989) which used the concentrated crude culture filtrate antigen of the mycelial phase is also specific for the identification of *P. marneffei.* This exoantigen test was applied to detect the precipitin bands in patient's sera (Viviani et al,1993). Thirteen sera collected serially from the same patient infected with *P. marneffei* gave positive results 2 months after the initial treatment but twice the concentration of the sera had to be used. Later, it was used to detect antigens and antibodies of *P. marneffei* in serum specimens of AIDS patients by Imwidthaya et al (1997). In addition, Kaufman et al (1996) also used immunodiffusion and latex agglutination test to diagnose antigenemia during *P. marneffei* infection in 10 and 13 of 17 patients, respectively. In their study, whole yeast cells and yeast culture filtrate antigens were used as antigens. These highly specific tests did not cross-react with antibodies from 6 serum samples containing cryptococcal antigen or 6 urine specimens positive for *Histoplasma* polysaccharide antigens. Indirect immunofluorescent antibody

test (IFAT) was reported by Yuen et al (1994) using germinating conidia and yeast-hyphae form as antigens. Patients with penicilliosis marneffei had higher titer than other patients with fever and healthy subjects with a cut–off of the dilution 1:40 or lower for IgG. This test was also performed to detect yeast-like cells of *P. marneffei* in human tissue or in simulated tissue by using rabbits antiglobulins that were immunized with whole yeast-like cells and yeast culture filtrate antigens (Kaufman et al,1995). Both types of antiglobulins reacted with yeast-like cells of *P. marneffei* and with *H. capsulatum*, but not with their respective mycelial forms. The antiglobulins also failed to react with the yeast and hyphal forms of a variety of other heterologous fungi. The cross reaction with *H. capsulatum* could be cleared by adsorptions specific antiglobulin with yeast cells of *H. capsulatum*. Immunohistochemical examination with monoclonal antibody EB-A1 which had been raised against *Aspergillus* galactomannan, having at least one epitope common to *P. marneffei* and *Aspergillus* species, could also be used to detect *P. marneffei* in the biopsy specimens (Pierard et al, 1991; Estrada et al, 1992).

Recently, Vanittanakom et al (1997) studied immunogenic protein antigens of *P. marneffei* yeast-form secreted into culture medium during the deceleration and early stationary phases of growth by using immunoblot assay. Thirty out of 33 sera from penicilliosis marneffei with AIDS patients recognized one or more of the four major proteins in sizes of 200, 88, 54, and 50 kDa. Their recognitions by sera were 72.7%, 93.9%, 60.6%, and 57.6%, respectively. The band of 88, 54 and 50 kDa gave strong reactions with about a half of serum samples. In one serum derived from an AIDS patient, reactivities to the 50 and 54 kDa proteins could be strongly detected two months before the definite diagnosis by fungal culture. Further studies in different groups of patients showed that the 50 and 54 kDa antigens seem to be specific for *P. marneffei*. In contrast with the other study which reported that a specific *P. marneffei* antigen of 38 kDa secreted in the culture broth of yeast- and mold-form for 6 weeks could be recognized by 45% of sera derived from AIDS patients with culture-confirmed *P. marneffei* infection. However, the reactivities could also be seen in patient's sera with other mycoses such as Cryptococcosis and Candidiasis (Chongtrakool et al,1997). Jeavons et al (1998) described identification and purification of specific cytoplasmic antigens of *P. marneffei* by using liquid isoelectric focusing (IEF). The collected fractions were subjected to sodium dodecyl sulfate-

polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. Three antigens of 61, 54, and 50 kDa were recognized by IgG antibodies from individual of 21 *P. marneffei* infected AIDS patients. The recognitions were about 86%, 71%, and 48%, respectively. Although, several studies have identified a number of antigenic determinants in secreted antigen preparations from *P. marneffei*, these antigens have not been effectively characterized. This study was focused on the 88 and 50 kDa antigens which were secreted and were strongly reacted with penicilliosis marneffei patients' sera.

The major objectives of this study are:

1. To purify highly immunogenic antigens (88 and 50 kDa) from the yeast culture filtrate antigens of *P. marneffei*.

2. To characterize their glycoprotein properties, lectins binding such as concanavalin A and wheat germ agglutinin, characteristic indicatives of mannoprotein or glucoprotein and N-acetylglucosamine or N-acetylneuraminic acid, respectively.

The results will be helpful in understanding the structural components of these highly immunogenic antigens (88 kDa and 50 kDa) of *P.marneffei* which may have some roles in the progress or protective immunity of the disease.