I. INTRODUCTION

Cryptococcus neoformans is an ubiquitous and an important opportunistic yeast that causes cryptococcosis in humans and animals worldwide, especially in immunocompromised patients, such as those with AIDS or organ transplant. In addition, a significant proportion of infections occurs in immunocompetent hosts (Mitchell et al., 1995). Cryptococcosis, like many other fungal infections, is thought to begin with inhalation of airborne fungi from an environmental source. It has been reported that the most important natural source is weathered pigeon dropping or soil contaminated with avian excreta. Data reported in the literature indicate that *C. neoformans* can be found as a transient commensal organism on humans or as an incidental colonizer in the respiratory tract or on the skin of healthy subjects or even patients with bronchopulmonary disorders (Garcia -Hermoso et al., 1999).

C. neoformans is an encapsulated spherical to oval budding yeast. The size of the polysaccharide capsule in *C. neoformans* varies according to the strain and the culture conditions. Most *C. neoformans* isolates have medium-sized polysaccharide capsules that result in cell diameters ranging from 4 to 10 μm. Some poorly encapsulated strains have diameters of only 2 to 5 μm. In heavily encapsulated strains recovered from infected tissues, the diameter of the cell can be as large as 80 μm (Casadevall and Perfect, 1998). This organism can also undergo sexual reproduction, and since it is a basidiomycete (*Filobasidiella neoformans*), it forms basidiospores. Sexual reproduction appears to occur much less frequently in nature than asexual or vegetative reproduction. The sexual spores (basidiospores) are approximately 1.8 to 3 μm in diameter and result from crosses of the α- and a- mating types on an appropriate medium (Buchanan and Murphy, 1998). *C. neoformans* grows well at 37 °C, but higher temperatures inhibit growth and kill the fungus. It tolerates a pH range of 4 to 7.5, but growth is significantly inhibited at a higher pH (Casadevall and Perfect, 1998).

Based on the antigenic composition of its polysaccharide capsule, biochemical, morphological and genetic characteristics, *C. neoformans* has been subdivided into two varieties and five serotypes, namely, *C. neoformans* var. *neoformans* (serotypes A, D and AD) and *C. neoformans* var. *gattii* (serotypes B and C) (Kwon-Chung and Bennett, 1992). Serotype AD, was subsequently identified using a panel of reciprocally absorbed antisera. The structure of the capsular polysaccharide responsible for the antigenic reaction has also been investigated and was found chemically different in the five serotypes. Serotype AD, however, was not recognized by other authors who classified reference strains previously serotyped as AD as A or D using an immunofluorescence assay with the monoclonal antibody E1 (Dromer et al., 1993). Differences between the two varieties with regard to pathogenicity and geographical distribution have been described. *C. neoformans* var. *neoformans* is responsible for most cases of cryptococcosis in immunocompromised host, especially in AIDS patients (Baro et al., 1999) and has a worldwide distribution. It has been reported that the isolates of *C. neoformans* that infect AIDS patients are predominantly of serotype A (Kwon-Chung and Bennett, 1992). Infections due to strains belonging to serotype D are more prevalent in certain geographic areas, including France, Italy, and Denmark and its infections are more likely than serotype A infections to occur in older patients, to result in skin involvement, and to be associated with corticosteroid therapy (Franzot et al., 1998). *C. neoformans* var. *neoformans* has occasionally been isolated from a variety of natural sources including peach juice, milk, fruits, and vegetables but has been primarily associated with avian excreta and, to a lesser extent, with soil contaminated with avian excreta (Ajello, 1958 ; Kwon-Chung and Bennett, 1992 ; Levitz, 1991 ; Rippon, 1988). *C. neoformans* var. *neoformans* has been most frequently isolated from weathered pigeon excreta and occasionally from the guano of other avian species, for example, chicken, parrots, sparrows, starlings, turtle dove, and canaries (Kwon-Chung and Bennett, 1992 ; Levitz, 1991 ; Rippon, 1988).

In contrast to *C. neoformans* var. *neoformans*, the distribution of disease caused by *C. neoformans* var. *gattii* is restricted to semitropical and tropical regions distribution and has been recovered from the environment in plant detritus of closely related eucalyptus species, such as *Eucalyptus camaldulensis* (river red gum) and *E.*

tereticornis (forest red gum) (Ellis and Pfeiffer, 1990) Recently, data showed that three additional eucalypts, namely, *E. blakelyi* (Blakely's red gum), *E. gomphocephala* (tuart), and *E. rudis* (flooded gum), also serve as abodes for *C. neoformans* var. *gattii* (Pfeiffer and Ellis, 1997). *C. neoformans* var. *gattii* is predominantly pathogenic and can be found in patients with no underlying immunosuppression, but it is rarely the cause of disseminated infection in patients with AIDS, even in endemic area (Montenegro and Paula, 2000 ; Casadevall and Perfect, 1998).

Recently, it has been proposed that serotypes A and D be given separate variety status, *C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *grubii* (serotype A), based on phenotypic and genetic difference between them (Franzot et al., 1999).

Several typing approaches have been used in epidemiological studies, including serotyping (Chen et al., 1996). Although some antigenic variation has been demonstrated within the capsular polysaccharide, this conventional typing system (serotyping) is not sufficiently sensitive to discriminate between individual strains (Cherniak et al., 1994) and determine the geographical origin of a cryptococcal isolates. (Dromer et al., 1994). The identification of medically important fungi is based on morphological and physiological characteristics and is often difficult and time-consuming. Because the frequency of and mortality due to opportunistic mycoses are increasing among patients with AIDS, hematologic malignancies and transplants, there is an urgent need for improved method to identify fungal pathogens. Novel molecular approaches for the genetic identification of fungal strains and species appear to offer advantages of simplicity, speed, and accuracy (Meyer et al., 1993a). Several recent reports have established that genotypic variation in *C. neoformans* can be identified by molecular techniques, such as pulsed-field gel electrophoresis (Kwon-Chung et al., 1992 ; Perfect et al., 1993), restriction fragment length polymorphism (RFLP) (Currie et al., 1994 ; Polacheck et al., 1992) and PCR-fingerprinting or random amplified polymorphic DNA (RAPD) (Crampin et al., 1993 ; Mayer et al., 1993b).

Fungal infections are an important problem in medicine nowadays. The rate of fungal infection in Thailand is increasing, not only because of environmental

factors that support the growth of several fungi, but also because there are an increasing number of immunocompromised patients, particularly those with AIDS. *Cryptococcus neoformans*, the organism causing cryptococcosis, was first reported to be pathogenic to man in 1894 and was isolated from pigeon manure in 1955 (Rippon, 1982). The first reported case of cryptococcosis in Thailand was recorded in 1960 (Satitnimarnkarn et al, 1960). In 1992 at Siriraj Hospital in Bangkok, Thailand, the number of cases of cryptococcosis incresed to 30, of which 27 were AIDS related and in 1993 there were 57 cases with 49 AIDS related (Prariyachatigul et al.,1996). In Chiang Mai, there were approximately 21% cryptococcosis patients, which were associated with AIDS. Cryptococcosis was found to be the third most common opportunistic disease in AIDS patients after tuberculosis and pneumocystic carinii, subsequently (Division of Epidemiology, Ministry of Public Health,1999).

In 2001, Khayhan had studied on serotype and PCR-fingerprints using a $(GACA)_4$ primer of 132 *C. neoformans* isolates from clinical and environmental sources in Chiang Mai by PCR-fingerprinting with $(GACA)_4$ primer. All clinical and environmental isolates were identified as *C. neoformans* var. *grubii* serotype A, except one strain belonged to serotype AD. Most clinical and environmental strains (130 of 132), including one isolate of *C. neoformans* serotype AD showed the identical PCR-fingerprinting pattern (designated as profile AI). Profile AII was observed in two environmental isolates (Khayhan, 2001). In his study, PCR-fingerprinting with a $(GACA)_4$ primer distinguished the four standard serotype of *C. neoformans*. Fewer variations of PCR-fingerprinting profiles in this study may be due to the fact that isolates from these restriction areas in Chiang Mai were less heterogeneous. On the other hand, using only one primer may not be enough to discriminate among these isolates.

In this study, RAPD with three arbitrary primers was used as DNA analysis of *C. neoformans* isolates from both clinical and environmental sources in Chiang Mai. RAPD analysis by an arbitrary primer method is simple and quick and does not require exhaustive characterization of the sequence of genomic DNA or the performance of various isotype techniques (Yamamoto et al.,1995). Moreover, this techniques has been used to produce highly discriminative and reproducible fingerprints for isolates of *C. neoformans* (Crampin et al.,1993).

Pigeon excreta is the saprophytic source most commonly associated with *C. neoformans*, but the fungus has also been isolated from a variety of other avian excreta, including that of dove (Tharavichitkul et al., 1973), canaries (Griseo et al., 1995), parrots (Lopez-Martinez and Castanon-Olivares, 1995), chickens (McDonough et al., 1961 ; Swinne et al., 1986), and other species. Although cryptococcosis is one of the most common opportunistic infection among AIDS patients living in Chiang Mai, there was no information on the environmental sources of this disease. Thus, a study on the domestic environment of cryptococcosis patient is also set up to determine sources of *C. neoformans* in the patients' homes area. The possible risk factors for cryptococcosis such as bird or chicken feeding will be recorded. Droppings of birds or chicken will be collected for the isolation of *C. neoformans*.

The isolates from these avian droppings and their clinical isolates are studied by RAPD analysis with three arbitrary primers. RAPD patterns of environmental isolates were compared with those of clinical isolates to determine their relations.

The major objectives of this study are:

1. To study genetic diversity and epidemiology of clinical and environmental isolates of *C. neoformans* in Chiang Mai, clinical isolates from Khon Kaen and Japan by RAPD analysis with three arbitrary primers (R28, OPH-02 and OPH-20).
2. To study sources of cryptococcosis from patients' homes in Chiang Mai.

These results will be useful for further studies on epidemiology and genetic diversity of clinical and environmental isolates of *C. neoformans* in Chiang Mai.