Thesis Title	Biological and Molecular Characterization of Pythium insidiosum the Etiologic Agent of Human Pythiosis
Name	Rapee Borisooth
Degree	Master of Science (Microbiology)
Thesis Supervisory Committee	Angkana Chaiprasert, Dr.rer.nat. Wanchai Wanachiwanawin, M.D.
Date of Graduation	25 November B.E. 2536 (1993)

ABSTRACT

Pythium insidiosum is a newly described and illustrated human pathogenic fungal species. It is the etiologic agent causing pythiosis, a cosmopolitan disease of horses, cattle, dogs, fish, and cats. Human pythiosis was first reported from Thailand. According to the clinical symptoms of human pythiosis, the patients could be classified into 2 groups. The first group of patients had cutaneous infection (cutaneous pythiosis) and some had progressive systemic *Pythium* infection (systemic pythiosis). Most of them had an underlying disease of thalassemia hemoglobinopathy syndrome. Systemic pythiosis cases developed severe clinical features including arterial occlusion and gangrene. Radical amputation was the only method to ensure survival of the patients, but some patients died. Another group of patients had corneal

Pythium infection (keratic pythiosis). One of them had thalassemia hemoglobinopathy syndrome (1/5). The therapeutic keratoplasty followed by giving eye-drop of some antifungal agents was recommended for keratic pythiosis. However, some patients recieved evisceration therapy.

The biological and molecular characterizations of *P. insidiosum* isolated from 2 group of patients were compared in this study. The study revealed that all 16 strains of *P. insidiosum*, 7 strains isolated from keratic pythiosis cases and 9 strains isolated from systemic pythiosis cases, could be induced to produce asexual reproduction in laboratory. Each strain could produce vegetative hyphae, reproductive hyphae, zoosporangia and zoospores similary. And the size of these structures were significantly not different.

Since most of systemic pythiosis cases were thalassemic patients who had iron level higher than normal persons, so the effects of iron on growth of *P. insidiosum* were studied. Two strains of *P. insidiosum* isolated from 2 systemic pythiosis patients were grew significantly not differently in the iron media and iron-free(iron-chelator excess) media. Both strains were also grew not differently in thalassemic and normal sera media. This study could be summarized that the iron overload status in thalassemic patients might not be the important predisposing factor of the disease in these patients.

The ribo-typing of both isolated groups were compared by using Southern blot DNA hybridization with rRNA-gene of yeast, *Saccharomyces carlsbergensis.* The ribo-typing of both

isolated groups had 4 types for *Eco* RI digested-DNA and 7 types for *Pst* I digested-DNA. There was no specific DNA-hybridization patterns related to *P. insidiosum* strains that caused systemic pythiosis or keratic pythiosis.

In summary, *P. insidiosum* strains isolated from keratic pythiosis cases and systemic pythiosis cases had biological and molecular characterizations not differently.