VII. SUMMARY

1. By screening of the yeast-phase expression cDNA library of *Penicillium marneffei* with a fragment of *hsp70* gene (clone H3), twenty-one positive clones were isolated. A full length 2,204-bp *hsp70* cDNA clone, ph20, was isolated and characterized. An open reading frame of *hsp70* composed of 1,911 basepairs was delimited by the well-known start (ATG) and stop (TAA) codons. The polypeptide consisted of 636 amino acids and had a calculated molecular mass of 69.5 kDa and a pI of 5.03. The clone ph20 contained 96 and 201 bases of 5' and 3' untranslated regions, respectively.

2. The structure of *P. marneffei hsp70* was identified on both nucleotide and amino acid levels. The presence of all conserved sequence motifs of the Hsp70 family in deduced amino acids of *P. marneffei* gene suggests that this gene is indeed the Hsp70-encoding gene. Amino acids were aligned using ClustalW and a phylogenetic tree was constructed using MEGA3 program; these revealed deduced amino acids of *P. marneffei hsp70* that were highly similar to fungal cytosolic Hsp70, which are closely related to those of *A. nidulans*.

3. An interesting feature of the *P. marneffei hsp70* gene is the presence of a microexon that contains only 3 nucleotides. The coding region of a *P. marneffei hsp70* gene is interrupted by two sections of different base length (57 and 198 basepairs, respectively) that flank to the 3-nt microexon.

4. Southern blot analysis of the genomic DNA using *P. marneffei hsp70* fragment as a probe suggests that the genome of *P. marneffei* contains more than one copy of *hsp70* gene that encode cytosolic Hsp70s.

5. Northern blot analysis indicates that *hsp70* transcripts are accumulated in the conidia of *P. marneffei*. The downregulation of the *hsp70* transcript during the development from conidia to mycelial phase at 25°C indicates that *hsp70* is constitutively expressed during growth in natural conditions. The differential expression of *hsp70* during the transition phase from conidia or mycelial to yeast phase was observed. A slight upregulation of *hsp70* transcripts occurred at 1 h and remained high until 24 h after the temperature rose from 25°C to 37°C. Subsequently, the expression of this gene was downregulated at the yeast phase from 48 to 96 h. The slightly upregulation of *P. marneffei hsp70* transcripts during an early stage due to the temperature increase may play a role in cells survival during infection in the host body at 37°C.

6. In an experiment imitating normal homeostatic condition (37°C) and during simulated human fever (39°C), the *hsp70* transcripts were rapidly abundant upregulated within 30 minutes after the temperature rose from 37°C to 39°C. This result suggests that Hsp70 production may be a putative virulence factor of *P. marneffei* for cell survival during host response by heat shock at 38-39°C. However, the investigation of *hsp70* gene expression during severe heat shock condition (42°C) implied the cessation of *hsp70* mRNA synthesis. It revealed the limitation condition for biosynthesis of this fungus.

7. Splicing of putative introns in *P. marneffei hsp70* mRNA was studied by RT-PCR using primers flanking the introns, and confirmed by DNA sequencing. The results showed an abundant band corresponding to the mature mRNA and a small population of unspliced *hsp70* mRNA created during mycelial-yeast-phase transition. Furthermore, the DNA sequence of the intron-containing fragment showed that it contained only intron II of *P. marneffei hsp70* gene. It is likely that inefficient splicing of the intron, which lies downstream from microexon, may be a characteristic of transcripts that contain microexon in several organisms including *P. marneffei*. The absence of a smaller intron I (57 bp) in this study suggests that splicing may occur more frequently at the smaller fragment of introns that flank the microexon.