VII. SUMMARY

7.1. Phagocytosis and killing activity of mouse macrophage J774.1 against conidia of pathogenic *Penicillium marneffei* was examined in comparison with non-pathogenic *Penicillium citrinum in vitro*. Both pathogenic and nonpathogenic *Penicillium* isolates used in this study showed no difference in the percentage of phagocytosis by J774.1. However, the phagocytic indices of *P. marneffei* were higher than those of *P. citrinum* after co-incubation at 60 min. This result revealed that *P. marneffei* was more susceptible to macrophage J774.1 cells than *P. citrinum*. In addition, the conidia of *P. marneffei* seemed to be more resistant to being killed by macrophages than those of the non-pathogenic fungus, *P. citrinum*.

7.2. The potent virulent genes of *P. marneffei* were isolated and characterized as follows:

A) Copper, zinc superoxide dismutase gene, designated as *sodA*, was isolated and characterized. The putative SodA polypeptide consisted of 154 amino acids and exhibited a significant level of similarity to other fungal Cu, Zn SODs. Moreover, the multiple sequence alignment showed many conserved sequence positions among all species.

B) cDNA (*gapdh*) and genomic clones that encoded the GAPDH were isolated and characterized. The *gapdh* cDNA contained a full length of 1,011 nucleotides. The deduced 337 amino acid sequence of the *gapdh* clone showed significant identity to the GAPDHs from other fungal species (87–73%). Phylogenetic analysis of the GAPDH amino acid sequences relating to the position of introns indicated that GAPDH could be useful for the determination of evolutionary relationships in fungi.

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C) Catalase (*cat*) and calmodulin (*cam*) genes of *P. marneffei* were partially isolated and characterized. The sequences of both genes showed high identity to *cat* and *cam* genes of other fungi, respectively. More characterization and investigation of their roles in virulence of *P. marneffei* will need further studies.

7.3. Total RNA samples were collected from different phases, phagocytosed conidia and conidia control at 2, 4 and 8 h of infection. The appropriate primers were designed from the sequences of *sodA*, *cpeA*, *gapdh*, *cat*, *cam, hsp70* and *acuD* genes of *P. marneffei* for RT-PCR analysis. Then, duplex RT-PCR was performed for differential gene expression. Part of the 18S rRNA sequence was used as an internal control in the reaction. Two RT-PCR-independent experiments were done in duplicate. A relative expression level of each gene was calculated from the ratio of the band intensity between the analyzed gene and control.

7.4. Differential gene expression by RT-PCR analysis revealed that *sodA* and *cpeA* transcripts accumulated in conidia, but their expressions were downregulated in the mycelial phase. Interestingly, the expressions of both genes were upregulated both in the yeast phase as well as during macrophage infection. The differential expression of *gapdh*, *acuD* and *hsp70* in different phases and during macrophage infection was not statistically significant. Low abundant transcripts of *cat* and *cam* genes were found in only conidia of *P. marneffei*. Therefore these latter two genes were excluded in the next gene expression experiment.

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7.5. By Northern blot analysis, the expression results of *sodA* and *cpeA* correspond to those from the RT-PCR analysis. More upregulation could be found in yeast than in conidial and mycelial phases. Some different results were obtained in the three genes, *acuD, gapdh* and *hsp70.* Increased expression of *acuD* was observed in the yeast phase. For *hsp70* and *gapdh*, the transcripts were accumulated in the conidia, with low expressions in both mycelial and yeast

phases. From these results, Northern blot analysis may be suitable to detect slightly differential gene expression that could not be seen by RT-PCR method.

7.6. In an early stage the fungal response to macrophage environment, the transcripts of stress response genes, *sodA* and *cpeA* were upregulated and constitutively expressed until the late stage of infection to protect fungal cells from oxidative radicals produced by the macrophage. In addition, *hsp70* expression was maintained during inside macrophage.

7.7. The increase of *acuD* transcript during macrophage infection indicated the induction of an alterative carbon metabolism for fungal adaptation to poor-glucose environments inside the macrophage while the expression of *gapdh* gene that was involved in the glycolytic pathway was repressed since an early 2 h of infection.

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