

CHAPTER VII

Summary

- 7.1 *P. marneffei sakA* and *atfA* genes were isolated and characterized. The nucleotide sequences of *sakA* and *atfA* transcripts were translated to amino acids. The deduced amino acid sequences from both transcripts revealed high homology to proteins in the stress Hog1/Spc1/p38 MAPK family and the bZip transcription factor family in fungi, respectively.
- 7.2 Expression analysis of *P. marneffei sakA* and *atfA* genes by RT-PCR demonstrated that *sakA* gene was up-regulated in conidia but not in mycelia and yeast cells under heat stress at 39°C and 1 mM H₂O₂ stress for 1 h, whereas *atfA* gene expression did not increase in any conditions.
- 7.3 Modification of split marker method for target gene deletion in *P. marneffei* strain F4 was successful, but the transformation efficiency was quite low. In PCR-based method, four transformants were grown on selective agar after *sakA* mutant generation. One of them showed homologous recombination at the target site. For *atfA* mutant, combination of PCR-based and plasmid-based methods resulting in five of transformants and one of them revealed homologous recombination at the *atfA* locus. Both *sakA* and *atfA* mutants contained only single copy of selectable marker (*hph*) gene.
- 7.4 Characterizations of *P. marneffei sakA* and *atfA* mutants compared to the wild type and complemented strains demonstrated that:
 - A) The *sakA* gene but not *atfA* gene played a role in conidial production (asexual development) in *P. marneffei*.

- B) The *sakA* gene participated in transition from mold to yeast cells in *P. marneffeii* *in vitro* (BHI and SDB) and inside mouse and human macrophages.
- C) Both the *sakA* and *atfA* genes were not involved in osmotic and UV stress responses in *P. marneffeii*.
- D) The *sakA* gene was involved in chitin deposition along the hyphae of *P. marneffeii* but deletion of this gene did not affect cell wall integrity under calcofluor white stress at 25°C. However, *sakA* was required for cell membrane stress (SDS) response at both 25°C and 37°C. The *atfA* gene did not play a role in chitin deposition and cell wall integrity under cell wall stress, but similar to *sakA*, this gene was participated in cell membrane stress (SDS) response of *P. marneffeii*.
- E) The *sakA* gene was associated in response against oxidative stress from inorganic peroxide H₂O₂ and organic hydroperoxide *t*-BOOH but not superoxide (menodione), whereas the *atfA* gene was involved in response to only *t*-BOOH but not H₂O₂ and menodione.
- F) The *sakA* gene played a role in heat stress response at 39°C of *P. marneffeii*, whereas the *atfA* gene was not involved in thermal stress response at both 39°C and 42°C. However, heat stress condition at 42°C could activate the relative expression levels of both *sakA* and *atfA* genes.
- G) Both *sakA* and *atfA* genes were required for survival of *P. marneffeii* conidia inside mouse (J774) and human (THP1) macrophages cell lines. Therefore, these two genes might associate with virulence of *P. marneffeii*.