VI. SUMMARY

1. Extracellular protein antigens secreted from the yeast form of *P. marneffei* 391H and 302BM during deceleration and early stationary phase of growth were analyzed by SDS-PAGE and immunoblot assay. The most highly immunogenic proteins had relative molecular masses of 88 and 50 kDa and strongly reacted with pooled sera from 10 AIDS patients infected with *P. marneffei*. The 88 kDa antigen could also be recognized by IgG antibody in sera from healthy persons or from pre-immune rabbit serum.

2. Protein purification was performed by using the preparative gel electrophoresis technique. The 88 kDa antigen was eluted continually in the elution buffer fraction numbers 160 to 210 by using Prep-cell preparative gel electrophoresis. The 50 kDa antigen was purified by using Mini-Protean II preparative gel electrophoresis followed by CBB staining, gel excision and electro-elution. Their molecular weight and antigenicity were confirmed by SDS-PAGE/ Western blot analysis.

3. Further purification of partially purified antigens of 88 and 50 kDa was conducted by using the two-dimensional gel electrophoresis technique. The pI values of these antigens were approximately $\leq$ 4.5 to 5.6 and $\leq$ 4.5 to 5.1, respectively, obtained by matching the sample gels with the standard gel on two-criteria. One was the absolute spot position and other was relative spot positions and intensities. However, these results could not be used to specify whether or not the antigens were contaminated with one or more species of proteins because the samples had streaked on the second dimension gel (SDS-PAGE).

4. Glycoprotein analysis of purified 88 and 50 kDa antigens after separating on two-dimensional gel and transferring onto nitrocellulose membrane showed that both of them were glycoproteins. The 88 kDa antigen was a large molecule with a large carbohydrate moiety, whereas the 50 kDa antigen had a very small carbohydrate moiety.

5. Characterization of these glycoproteins on the nitrocellulose membrane by using lectin binding showed that the 88 kDa antigen had higher affinity for Con A than the 50 kDa antigen. This result suggests that alpha-D-mannopyranoside or alpha-D-glucopyranoside residues were the major carbohydrate components of the 88 kDa antigen, whereas they were minor components of the 50 kDa antigen. Both glycoproteins did not bind with WGA, suggesting that their molecules may not have N-acetylglucosamine or N-acetylneuraminic acid residues or they may be present in trace amounts which could not be detected by this method.