CHAPTER V

DISCUSSION

The complexity of proteins can be analysed by means of determining their molecular weights through the SDS-PAGE technique.[82] Hence, the objective of this study is to investigate the possibility of components variation in *P. pseudomallei* by SDS-PAGE. This may contribute some information regarding to its compositions and taxonomy.

By means of SDS-PAGE technique, the variation of components among thirty six strains of *P. pseudomallei* were illustrated at molecular weights ranging from 12.6 to 56.0 kd. Not only visuallization,but also densitometer were used in order to detect the variation of those components. All strains were classified into six types according to the aforementioned criteria.

Although there were the bands at molecular weights of 56.0, 27.5 and 12.6 Kd in both Type I and Type VI. Type I was more intensity of bands at molecular weight of 12.6 Kd. If we used only the presence of bands at molecular weights ranging from 12.6 to 56.0 Kd as the criteria to classify these organisms,these 36 strains might be classified into four groups. That because Type I and Type VI would be the same group.

Remarkably, the band at molecular weight of 14.4 was found

only in Type IV, 14.5 was found in both Type II and Type V. The band at molecular weight of 19.9 was dominate in Type III more than Type IV.

There was no correlation between biochemical reaction, geographic distributed and six typed which classified by SDS-PAGE technique.

Culture medium and condition of growth, are the factors which influence the bacteria components. Hood et al[97] and Kerster et al[98] reported that growth of culture media variety had some visible effect on the overall patterns and were found quantitatively different when strains were harvested at various stages during their growth cycles. In our preliminary studies, there were many factors which influenced the composition of protein; such as culture medium, aging of microorganisms and methodology used in protein extraction. Therefore all strains were cultured in brain heart infusion broth at 37 °C for 18 hrs to limit the above influenced factors.

The patterns of sonic extract of *P. pseudomallei* were similar to that of *P. cepacia*. In addition, they have the same major component at the molecular weight of 57.1 Kd as shown in Fig 26. This may have some connection to the facts that the biochemical characters of these organisms are similar[36] and they are classified in RNA group II[26].

However SDS-PAGE tecnique can not demonstrate the antigenic property of these protein components, therefore immunoblotting analysis should be performed.

The sensitive staining assay such as India ink stain was used to locate molecular weight markers as well as the direct visuallization of resolved protein components on nitrocellulose paper (98). It was known that the India ink staining was more sensitive than Coomassie blue, amido black and fast green stain(98). Hence in this study, India ink staining was selected for visuallizing total protein bands of *P. pseudomallei* and also for that of molecular weight markers. Since the immunoblot assay possessed a sensitivity of protein detection at the level of 80 ng(98), the method was used in detection of sero-reacting component of *P. pseudomallei*. The visuallization method of these components could be either enzymatics or radioactive reaction(99). In this study, the enzymatic method or EIA (Enzyme Immuno Assay) was used because of its reliability, reproducibility, economy and safety from radioactive harzard(100).

In order to study the antigenicity among the species of *P. pseudomallei* by immunoblotting, 36 strains of sonic extract of *P. pseudomallei* (5 ug protein per lane) were reacted with a rabbit hyperimmune antiserum (immunoglobulin) raised against the reference - strain of *P. pseudomallei* NCTC 4845 at the dilution of 1:100.

For comparison, *P, pseudomallei*. NCTC 4845 was run on each gel and used the same batch of the rabbit antiserum. The result obtained by this method can detect proteins with molecular weights of 12.0 to 140.0 Kd. Immunoblotting revealed variable bands at molecular weights of 56.0, 15.0 and 14.4. In comparing these antigenic patterns of *P. pseudomallei*, eighteen out of thirty five strains were similar to the reference strain of *P. pseudomallei* and

were clasified as group A. The variation in the absence and presence of antigenic bands at molecular weights of 56.0, 15.0 and 14.4 were used in the classification of the other clinical isolates and seperated them into group B and C.

It is interested to note that the antigenic grouping by an Immunoblotting was coincided with SDS-PAGE typing. For example, strains of *P. pseudomallei* which classified by SDS-PAGE as Type I and Type II; Type IV; Type III, V and VI were immunoblotted as group A, B and C respectively.

However, it must be emphasized that our antigenic grouping was not completed at the present time because of the dependence on the pattern of immunological cross-reactivity between those isolation strains to *P. pseudomallei* NCTC 4845. The discrimination in the antigenic pattern can be greatly improved by examining the result obtained with multiple antibody probes, liked using antiserum, which raised against each type of *P. pseudomallei*. So that the antigenic specificity of each strain may be observed.

Earlier observation by Duodin et al[35], using immunoelectrophoresis showed the serotype of *P. pseudomallei* which were isolated from different countries, particularly Australia and South East Asia, and enably defined it into at least two serotypes. They suggested that *P. pseudomallei* has an antigenically heterogenous group. Such information might be used to differentiate among *P. pseudomallei* in order to from the basis of serotyping systems.

Attempts had been made to determine the basis of the cross reactivity seen in patients serum by serological assays. For this analysis we also included other member of this species such as *P. aeruginosa, P. cepacia, P. stutzeri, P. putida, P. maltophila* and other members of family of *V. cholerae, S. typhi, E. coli* and *S. aureus*. These organisms and *P. pseudomallei* were reacted with the pool of antisera againt *P. pseudomallei* reference strain and used normal rabbit antiserum as the control.

The immunostaining patterns showed many significant antigenic components in each strain. Although some background bands with normal rabbit sera appeared, it was possible that the normal rabbit sera might have nonspecific antibody to these organisms before immunization. However some antigenic bands in *P. pseudomallei.* were also seen in the other previously descibed bacteria at the same molecular weight site. These might be the cause of cross reacting phenomenon among the strains which could be used to answer the serologically cross reaction we observed previously.

Interestingly, we found at least six bands with molecular weights of 16.8, 20.7, 24.1, 107.0, 115.0 and 140.0 Kd., every of which were appeared only in every strains of the species of *P. pseudomallei.* These bands might be the common antigens of *P. pseudomallei* and might likely to be species specific. This could possibly be advantageous for the development of diagnotic kits, vaccine and further application in monoclonal antibody production.

Conclusions :

The sonic extract of 32 clinically isolated strains of *P. pseudomallei* was able to differentiate into six Types by silver stained SDS-PAGE. In addition to the intensity of the bands and the presence or absence of bands at molecular weights of 56.0, 27.5,19.9, 19.6, 14.5, 14.4 and 12.6 Kd were used as criteria for this typing. The thirty two strains could be typed as follows; 9 strains were in Type I; 6 strains were in Type II, 1 strain was in Type III,3 strains were in Type IV; 3 strains were in Type V and 10 strains were in Type VI. The significant correlation among the anatomic sites of infection, geographic locations, and SDS-PAGE typing was not found.

These clinically isolated strains were further studied by the immunoblot technique using hyperimmune antiserum aganist *P.pseudomallei* NCTC 4845. The visible antigenic bands allowed the classification of our 32 clinical isolates into 3 group; A,B, and C respectively. The correlation between the SDS-PAGE typing and the immunoblot grouping was noted. SDS-PAGE Types I and II were with in Immunoblot group A, Type IV was in group B, and Type III, V and VI were in group C.

In addition, other organisms such as *P. aeruginosa* ATCC 27853, *P. cepacia* JCM 5510, *P. stutzeri* JCM 5969, *P. putida* JCM 6160, *P. maltophila* JCM 3801, *V. cholerae* 569B, *S. typhi* NCTC 781, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 showed antigenic bands ranging from 12.0 to 140.0 Kd to which were commonly found in all of the *P. pseudomallei* isolates. Therefore, these antigens may be

responsible for producing cross reactions in serological testing among these organisms and *P. pseudomallei*.

Interestingly antigenic bands at the molecular weight to 16.8, 20.7, 24.1, 107.0, 115.0 and 140.0 Kd were found in common to all strains of *P. pseudomallei* but were absent from the other bacterial strains named above. These antigens may be species specific.