

ORIGINAL ARTICLES

Immunoblot Analysis to Demonstrate Antigenic Variability of Clinical Isolated *Pseudomonas pseudomallei*

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Pseudomonas pseudomallei (Ps.ps.) is the causative organism of melioidosis that affects mainly people in Southeast Asia and Northern Australia. Clinical manifestations of melioidosis vary from subclinical infection to fulminant septicemia and because of this wide clinical spectrum, the disease has been called "the great imitator" for every infectious disease.¹

In Northeastern Thailand, melioidosis is a major cause of community-acquired septicemia.² Approximately 60% of septicemic form and 40% of localized form of the disease are diagnosed according to established clinical criteria. However, there is little information regarding the role of strain differences and its relationship with clinical manifestation.¹ Recently, immunoblot has been utilized as a powerful tool for fingerprinting hospital pathogens.³ Therefore, it is the aim of this study to demonstrate antigenic variability of Ps.ps. isolated from various clinical specimens using immunoblot analysis and to use this approach to differentiate between septicemic and localized forms of this disease.

SUMMARY *Pseudomonas pseudomallei* (Ps.ps.) is the causative organism of melioidosis, and is widely distributed in Southeast Asia and Northern Australia. Clinical manifestations range from subclinical infection to fulminant septicemia. To demonstrate the antigenic variability of Ps.ps., 62 clinical isolates from 31 blood, 13 sputum, 9 pus, 3 urine and 6 body fluid culture specimens were studied by SDS-PAGE and immunoblotting. In SDS-PAGE, there were approximately 20 antigenic components with molecular weights ranging from 14 to 66 kilodaltons (KD) which suggested that there was antigenic variability among these 62 clinical isolates of Ps.ps. Attempts to correlate immunoblot profiles with clinical illness or sources of specimens were not successful but 6 common antigens were identified with molecular weight of 17.5, 21, 33, 34, 40 and 45 KD, respectively. Among these antigens, the 45 KD component was recognised by all patients' sera. Thus, the 45 KD protein antigen may be useful for the future approach in immunodiagnosis of melioidosis.

MATERIALS AND METHODS

A total of 62 cultures of *Pseudomonas pseudomallei* (Ps.ps.) from the Clinical Microbiology Laboratory Unit of Srinagarind Hospital, Khon Kaen University, Thailand, were assigned code numbers before they were analysed as unknowns. Of these specimens, 31, 13, 9, 3 and 6 were from blood, sputum, pus, urine and body fluid of the patients respectively. Thirty and 20 specimens came from septicemic melioidosis and localized melioidosis patients respectively. The remaining of specimens came from unidentified form of melioidosis.

Preparation of antigens

Ps.ps. antigen from 62 isolates were prepared according to the method of Barber *et al.*⁴ with modifications. Briefly, washed and acetone dried bacterial cells from each isolate were extracted in 0.15 M veronal buffer (pH 8.4). The extract was dialysed, lyophilised

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and stored at 4° C until further use. Protein and carbohydrate contents were assayed by methods of Lowry *et al.*⁵ and Kabat *et al.*⁶ respectively.

Preparation of mouse hyperimmune antisera

One hundred µg protein from each antigen extract was injected intraperitoneally with complete Freund's adjuvant into 10-12 weeks Swiss albino mice (purchased from the National Center of Experimental Animals, Mahidol University, Thailand). Two booster doses were given intravenously at 2 weeks interval.⁷ Mouse antisera were collected, pooled, lyophilised and stored at 4° C until further use.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblots

Ps.ps. extracted antigens at concentration of 200 µg/100µ g per lane

were analysed using 12% polyacrylamide gels in a discontinuous buffer system of SDS-PAGE.⁸ After electrophoresis at 60 mA, the protein profiles were stained with Coomassie blue or transferred to nitrocellulose membrane by a Transblot apparatus with a 15°C cooling system (LKB, Sweden) at 90 V for 2 hours. The nitrocellulose membrane was reacted with mouse hyperimmune antisera and alkaline phosphatase conjugated goat antimouse IgG using a mixture substrates of β-naphthyl phosphate and O-dianisidine tetrazotized according to previously described method.⁹ The specific staining band showed a red-purple colour. Normal mouse sera and conjugate control were also included as negative controls.

Patients' sera

Three sera samples from melioidosis patients with indirect hemagglutination (IHA) titers of > 5120,

2560 and 640, and 4 sera from non-melioidosis patients with IHA titres of less than 20 were used in this study. In order to study human immune response against the extracted antigens, immunoblots with phosphatase conjugated goat anti-human Ig were employed for immunoblot analysis.

RESULTS

The average of protein and carbohydrate contents of Ps.ps. extracts were 23.8 mg and 5.7 mg per one gram of acetone dried cells respectively. Using SDS-PAGE, at least 20 reproducible protein components with molecular weight ranging from 66-14 kilodalton (KD) were identified from 62 Ps.ps. extracts. There were slight differences among each isolate according to the SDS-PAGE profiles (Fig. 1).

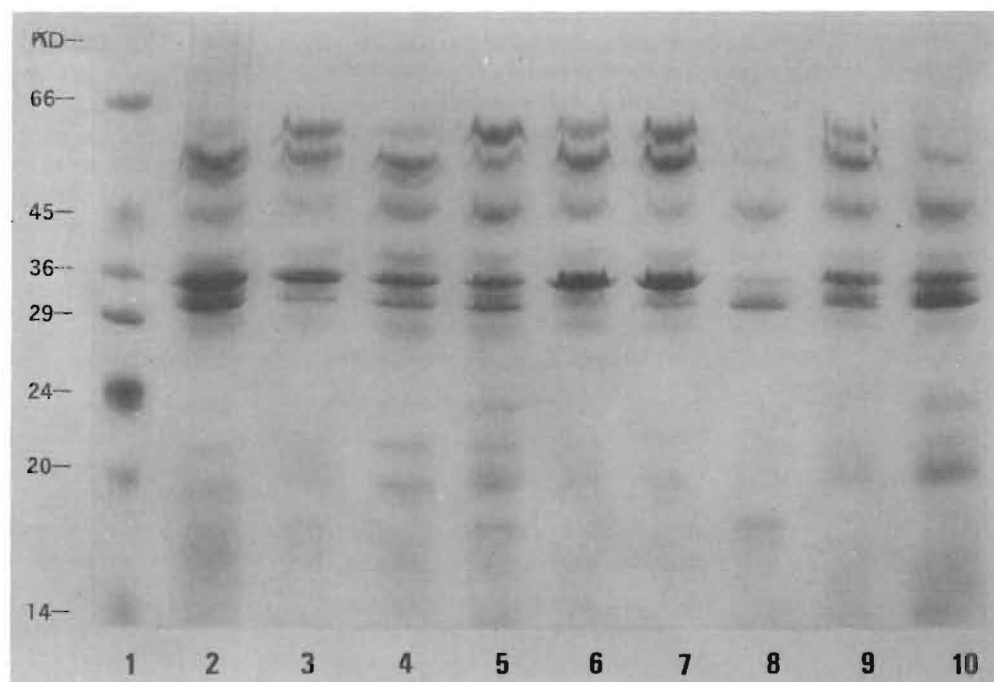


Fig. 1 SDS-PAGE profiles stained with Coomassie blue
 lane 1 Standard protein markers molecular weight of 66, 45, 36, 29, 24, 20 and 14 kilodalton (KD) respectively.
 lane 2- 10 Nine extracts of clinical isolated Ps.ps. from 3 septicemic melioidosis (lane 2,8-9) and from 6 localized melioidosis (lane 3-7, 10).

Analysis of the immunoblot profiles indicated that there was antigenic variability among clinical isolates of *Ps. ps.* (Fig. 2). Attempts to correlate immunoblot profiles with defined clinical illness or with sources of specimens were not successful. However, 6 common antigenic bands in each isolate with molecular weight of 45, 40, 34, 33, 21 and 17.4 KD were identified.

Further testings of the 6 common antigens were performed using melioidosis and non-melioidosis patients' sera. Preliminary results indicated that the 45 KD antigen was specifically recognised by all 3 melioidosis patients' sera but not with 4 non-melioidosis patients' sera (Fig. 3, Table 1). The other 5 antigenic bands showed variable degrees of specificity with the patients' sera.

DISCUSSION

This is the first report designed to explore the immunoblot fingerprinting of *Ps. ps.* extracts isolated from clinical cultures for differentiation between septicemic and localized forms of melioidosis. The results have shown variability among the isolates. When the criteria based on Burnie and Matthews³ was employed to compare the isolates run on the same gel by differences in the position, intensity or presence of each band, there should be at least three differences in antigenic bands before an isolate can be classified as distinct. Unfortunately, this technique could not be used to identify the relationship between isolated strains and clinical illness or sources of cultures.

In this study, 6 common antigenic bands are presented in each isolate. These bands can be recognised by the patients' sera, in which 45 KD band reacted commonly with all melioidosis patients' sera. The results from Wongratanacheewin *et al.* also revealed 7 antigenic components with molecular weight ranging from 44-19 KD that could be reacted with patients' sera (personal communi-

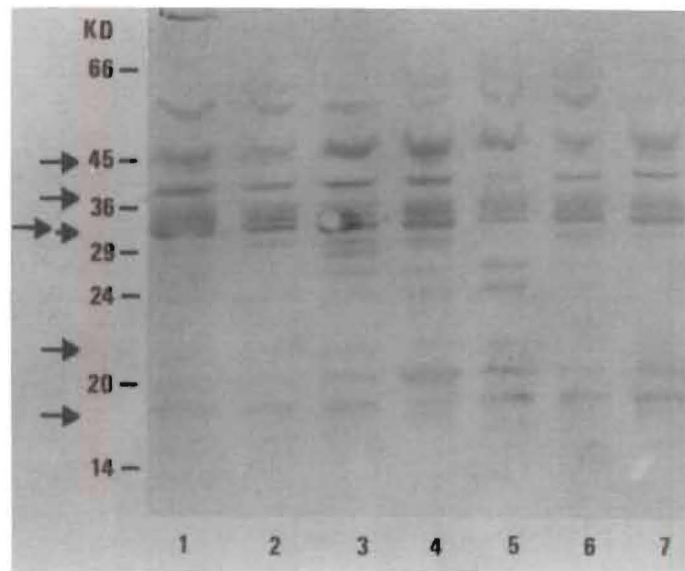


Fig. 2 Immunoblot profiles of 7 extracts from clinical isolated *Ps. ps.* (the same isolated as lane 2-8 respectively, in Fig. 1). The extracted antigens were reacted with mouse antisera against homologous antigen of *Ps. ps.* The 6 common antigenic bands are indicated with arrows at 45, 40, 34, 33, 21 and 17.5 KD.

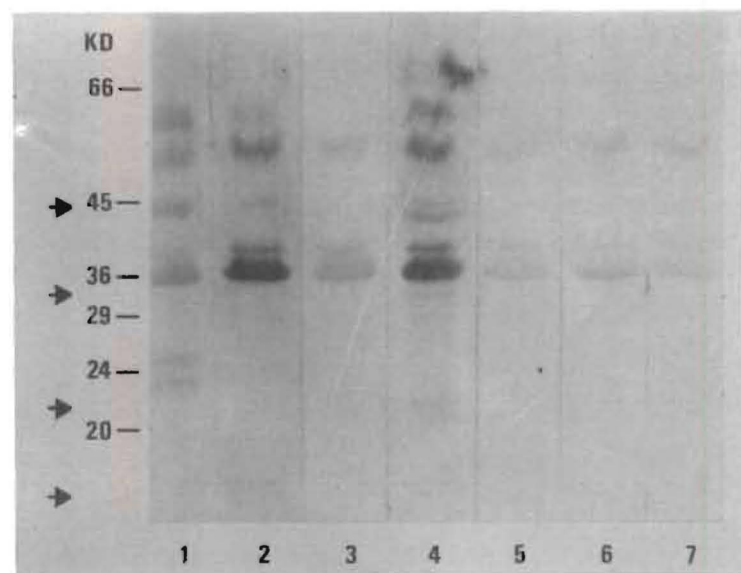


Fig. 3 Immunoblot profiles of single extract of clinical isolated *Ps. ps.* (the same isolated as lane 2 in Fig. 1). The extracted antigen was reacted with 3 melioidosis patients' sera (lane 1, 2, 4) and 4 non-melioidosis patients' sera (lane 3, 5-7). The 4 specific antigenic bands of *Ps. ps.* are indicated with arrows at 45, 33, 21 and 17.5 KD.

Table 1. The reaction and molecular weights of the 6 common antigenic bands of *Pseudomonas pseudomallei* in immunoblots with 3 melioidosis and 4 non-melioidosis patients' sera.

| Molecular weight (KD) | Reaction with melioidosis patients' sera * | Reaction with non-melioidosis patients sera * |
|-----------------------|--|---|
| 45 | 3/3 | 0/4 |
| 40 | 3/3 | 4/4 |
| 34 | 0/3 | 0/4 |
| 33 | 1/3 | 0/4 |
| 21 | 1/3 | 0/4 |
| 17.5 | 2/3 | 0/4 |

* number of positive results on tested sera

cation). Thus, the 45 KD protein antigen of Ps.ps. should be good immunogen in human and may be useful for immunodiagnosis of the disease. The approaches to confirm this finding should be further explored.

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