PROTEIN PROFILES OF FIELD ISOLATES OF *BACILLUS ANTHRACIS* FROM DIFFERENT ENDEMIC AREAS OF INDONESIA

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ABSTRAK

POERWADIKARTA, M.B. 1998. Profil protein *Bacillus anthracis* isolat lapangan dari beberapa daerah endemik di Indonesia. *Jurnal Ilmu Ternak dan Veteriner* 3 (1): 34-38.

Ekstrak protein dari 14 isolat *Bacillus anthracis* yang disonikasi berasal dari 6 daerah endemik antraks di Indonesia dianalisIS dengan teknik *sodium dodecyl sulphate polyacrylamide gel electrophoresis* (SDS-PAGE). Hasil penelitian menunjukkan bahwa profil protein dari seluruh isolat lapangan secara antigenik sedikit berbeda pada *band* protein dengan berat molekul 18, 37, 52, 65 dan 70 kDa. Profil protein tersebut juga bervariasi antara isolat lapangan dan galur vaksin. Variasi tersebut dapat memberi petunjuk bahwa sumber penularan antraks dapat berasal dari isolat yang sama atau berbeda.

Kata kunci : Profil protein , *Bacillus anthracis*, SDS-PAGE

ABSTRACT

POERWADIKARTA, M.B. 1998. Protein profiles of field isolates of *Bacillus anthracis* from different endemic areas of Indonesia. *Jurnal Ilmu Ternak dan Veteriner* 3(1): 34-38.

Sonicated cell-free extract proteins of 14 field isolates of *Bacillus anthracis* from six different endemic areas of Indonesia were analyzed by the use of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) methods. The protein profiles of each field isolate tested demonstrated slightly different at the protein bands with molecular weights of 18, 37, 52, 65 and 70 kDa, and varied between the field isolates and vaccine strains. The variation could provide clues to the source of anthrax transmission whether it was originated from similar strain or not.

Key words : Protein profiles, *Bacillus anthracis*, SDS-PAGE

INTRODUCTION

Bacillus anthracis is known as the causative agent of anthrax that has high risk to animals and human beings. In Indonesia, anthrax was first reported in animals in 1885 (SOEMANAGARA, 1958) and it has been recognized in several anthrax endemic areas. Anthrax outbreaks occurred in endemic areas not only affected animals but also human beings. Recent outbreaks of anthrax occurred in the regencies of Semarang, Central Java in 1990 (HARDJOUTOMO *et. al.*, 1990), and in Manggarai and Ngada of Flores island; East Nusatenggara in 1995. During the last anthrax outbreak, several species of domestic animals were affected, including: cattle, buffaloes, horses, pigs, goats, dogs, and olso human beings.

Anthrax vaccination programme of susceptible animals is recommended by Veterinary Service Officer (VSO) in order to stop the spread of the disease. Live spore vaccine of Sterne 34F2 strain is extensively used through out the country. Whereas antibiotics were frequently used to control human anthrax in endemic areas.

In recent years, an electrophoresis technique has been used to characterize the bacterial protein antigens (ELHAG and ALKAMRI, 1991; JONG *et.al.*, 1991; KUKOSCHKE and MULLER, 1991; XUDONG *et.al.*, 1996). This paper, presents preliminary studies of the protein profiles of field isolates of *B. anthracis* and their epidemiological significance.

MATERIALS AND METHODS

Bacterial isolates

Fourteen field isolates of *B. anthracis* were used in this study. They were isolated from surveillance samples in different endemic areas in Indonesia as indicated in Table 1.

Table 1. Field isolates of *Bacillus anthracis* used in this study

No.	Code of isolate	Originated	Location	Year isolated
1.	PK	Cattle	South Sulawesi	1984
2.	MR	Cattle	South Sulawesi	1984
3.	BN	Cattle	South Sulawesi	1984
4.	IR1	Pigs	Irian Jaya	1984
5.	BG	Goat	West Java	1985
6.	PW	Goat	West Java	1985
7.	KR	Lion	West Java	1992
8.	BKS	Goat	West Java	1985
9.	DP	Cattle	West Nusatenggara	1986
10.	SB	Cattle	West Nusatenggara	1989
11.	DKI1	Cattle	Jakarta	1982
12.	JT1	Cattle	Central Java	1990
13.	JT2	Soil	Central Java	1990
14.	JT3	Cattle	Central Java	1990

Preparation of cell free extract of *B. anthracis*

Each isolate was grown in brain heart infusion (BHI) broth, and incubated at 37°C for 16-18 hours supplemented with 10% CO_2. The broth cultures was then killed by peroxyacetic acid to a final concentration of 10%. Twenty four hours later, the cells were tested for their viability to confirm that all cells were killed. Cells were harvested by centrifugation at 12,000 rpm for 20 min. The cell pellet was washed 3 times with 0.02 M PBS (pH 7,4). The last cell pellet was resuspended in physiological saline, then was sonicated for 10 minutes. Each sample was split into aliquots and kept at -25°C until the next step.

The protein content in each sample suspension was determined by the modified of Lowry methods protein assay (HARTREE, 1972). Protein standard curve was prepared with bovine serum albumin (BSA), and the protein content was determined by this curve.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed as described by LAEMMLI (1970) with slightly modified: 12% separating gel of acrylamide:bis-acrylamide (30%T, 2.65% C) in 0.375 M Tris HCl, pH 8.8 and 0.1% (w/v) of SDS and 5% stacking gel in 0.125 M Tris HCl, pH 6.8 and 0.1% (w/v) of SDS. Each sample was dissolved with an equal volume of sample buffer (10% mercapto-ethanol, 4% SDS, 0.2% bromophenol blue, 20% glycerol in 100 mM Tris-HCl, pH 6.8). This sample was put in a boiling waterbath for 2 min. Twenty five microlitre (ul) of protein sample was filled into each well of the stacking gel. A constant current of 10 mA for 6 hours or until the dye front reached the lower edge of the gel. The gel was left off and then stained with coomassie brilliant blue for 4 hours, and photographs were taken after destaining.

The molecular weight of each band of protein sample was determined by compairing with molecular weight markers of 68-14 kDa (Sigma). Relative electrophoretic mobility (Rf) values were used for molecular weight estimation (HAMES, 1987). The Rf of protein was calculated from its migration distance from the top of the gel to the centre of the protein band divided by migration distance of Bromophenol blue tracking dye from the top of the gel. The Rf values were plotted against the known molecular weights as an ordinate on semi-logarithmic paper. The molecular weight of unknown protein was estimated from the calibration curve.

RESULTS AND DISCUSSION

Cell-free extract of 14 of *B. anthracis* isolated from six different endemic areas of Indonesia were analyzed by SDS-PAGE for their protein profiles. The result of protein bands of each cell- free protein extract of *B. anthracis* isolates are shown in Figure 1 and Figure 2. SDS-PAGE gels demonstrated wide range of molecular weights from 86 to 18 kDa. The main protein antigens were eleven bands with molecular weights of 86, 81, 70, 62, 52, 48, 46, 41, 37, 26 and 18 kDa (Table 2). The protein bands which were not detected in Sterne antigen were protein bands with molecular weights of 18, 46 and 49 kDa. Protein

profiles of field isolates were varied as shown in Table 2. Most of the field isolates frequently have the protein bands with molecular weights of 86, 81, 49, 46, 41 and 26 kDa, except for those of four isolates (KR, DP and SB, and JT1) which were isolated from West Java, West Nusatenggara and Central Java respectively, they had lost their protein band of 70 kDa molecular weight.

This study demonstrated that there were no apparent differences between protein profiles of 3 field isolates from South Sulawesi and 1 isolate from Irian Jaya. All isolates from these two areas had lost one of the main protein with molecular weight of 37 kDa. This might be from a similar source of the anthrax transmission. Field isolates from West Java had similar protein profiles, except for KR; they lost the protein bands of 65, 52 and 37 kDa molecular weights.

Two field isolates from West Nusatenggara had similar protein profiles with nine main bands: 86, 81, 65, 52, 49,46, 41, 37 and 26 kDa. These isolates did not have 2 protein bands with molecular weights of 70 and 18 kDa.

Field isolate from Jakarta (DKI1) did not have protein bands of 65, 37 and 18 kDa molecular weights. This isolate was similar in protein profiles to those from Central Java (JT2 and JT3). It was suggested that the isolate from Jakarta and 2 isolates from Central Java could be originated from a similar source. However, one isolate from Central Java (JT1) was slightly different from the other two (JT2 and JT3). JT1 isolate lost protein bands of 70 and 65 kDa molecular weights. This did not mean that JT1 isolate was quite different with the others. It must be envisaged to predict that the JT1 differs to the other isolates which only appeared by SDS PAGE.

From the study presented above suggested that all field isolates were similar, no apparent sub-type occurrence, but they had a slight variation in protein profiles. The protein profile variation merely occurred in protein band. These need to be investigated further in relation to their antigenicity and immunogenicity.

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Table 2. The main protein profiles of the field isolates of *Bacillus anthracis* from different endemic areas

Code of field isolate	Molecular weights of protein bands (kDa)										
	18	26	37	41	46	49	52	65	70	81	86
PK	+	+	-	+	+	+	+	+	+	+	+
MR	+	+	-	+	+	+	+	+	+	+	+
BN	+	+	-	+	+	+	+	+	+	+	+
IR1	+	+	-	+	+	+	+	+	+	+	+
BG	w	w	-	+	+	+	-	-	+	+	+
PW	w	w	-	+	+	+	-	-	+	+	+
KR	w	w	-	+	+	+	-	-	-	+	w
BKS	w	w	-	+	+	+	-	-	+	+	+
DP	-	+	+	+	+	+	+	+	-	+	+
SB	-	+	+	+	+	+	+	+	-	+	+
DKI1	-	+	-	+	+	+	+	-	+	+	+
JT1	+	+	+	+	+	+	+	-	-	+	+
JT2	-	+	-	+	+	+	+	-	+	+	+
JT3	-	+	-	+	+	+	+	-	+	+	+
STERNE	-	+	+	+	-	-	+	+	+	+	+

+ = clear band detected
w = weak band detected
- = no band detected

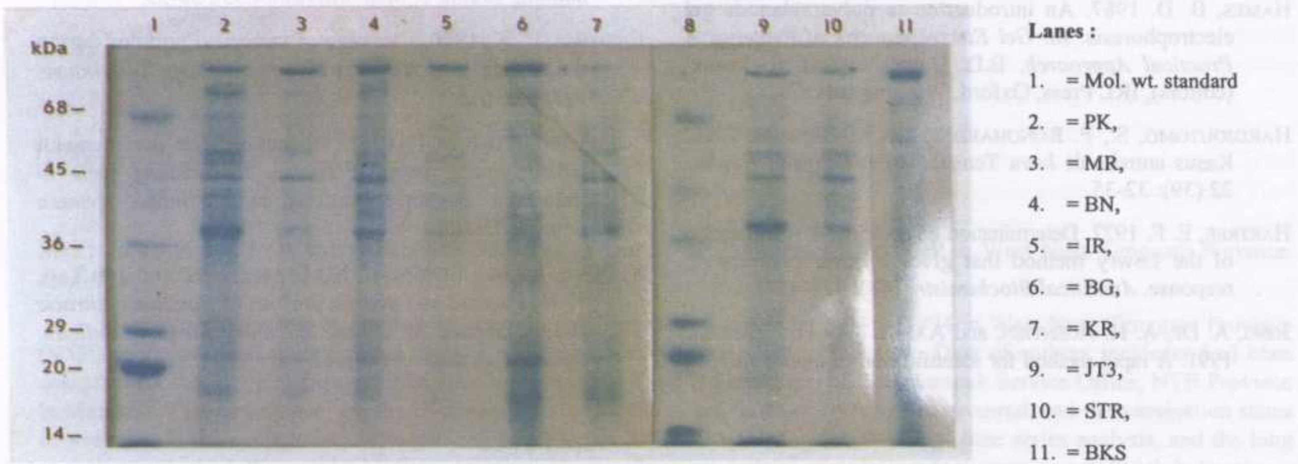

Figure 1. Protein profiles of the field isolates of *B. anthracis*

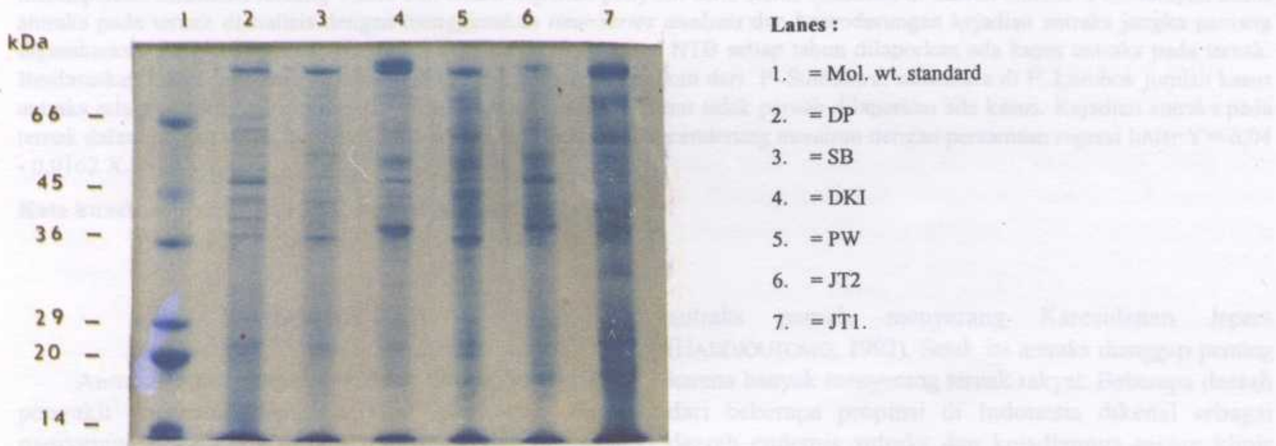

Figure 2. Protein profiles of the field isolates of *B. anthracis*.

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