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USE OF WESTERN BLOT TECHNIQUE FOR THE DETECTION OF THE IMMUNOGENIC DETERMINANTS OF CLOSTRIDIUM CHAUVOEI WHOLE CELLULAR ANTIGEN

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SUMMARY

Sodium dodcyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used for fractionation of the whole cellular antigens of C.chauvoei. The obtained results revealed the presence of 8 polypeptide bands which ranged from 205 to 18.5 KDa. A major protein band with a molecular weight of 49.5 KDa was identified. Application of the immunoblotting technique with the specific hyperimmune serum prepared against C.chauvoei whole cellular formalinized antigen in rabbits, showed the presence of some major antigenic epitopes which reacted strongly with the specific hyperimmune serum, mainly at 29.5 and 205 KDa.

INTRODUCTION

Disease caused by clostridial microorganisms are of economic impact to the livestock production. Black leg disease caused by C.chauvoei is one of the important clostridial diseases affecting cattle and sheep. Animals usually acquire the infection through contaminated feed, wounds and by inhalation of spores (Knott et al., 1985) . Many countries have adopted vaccination programmes to protect their animals against clostridial organisms mainly C.chauvoei, C.septicum and C.perfringens types B. and D. These vaccines are of either bivalent or polyvalent nature (Kerry and Craige, 1979).

Western blot technique is a powerful tool of protein analysis that involves transfer of protein separated by gel electrophoresis to a nitrocellulose membrane and immunoblotting analysis using specific antisera (Burnett, 1981) The present study was undrtaken to define th protein profile of the whole cellular antigen o C.chauvoei and to locate specifi immunodeterminants which may play a majo role in the immune response against black le disease caused by C.chauvoei.

MATERIAL AND METHODS

Cultivation and preparation of C.chauv whole cellular Antigen for gel electrophoresi C.chauvoei lyophilized strains No. 3121 & 41 (ATCC)* were reconstituted in freshly prepa

* ATCC: American Type Culture Collection

cooked meat broth and incubated anaerobically for 48 hours at 37°C. The cultures were examined for purity by subculture onto 10% sheep blood agar plates , incubated both aerobically and anaerobically and by Gram's stain. The purified cultures were reinoculated into brain heart infusion broth, incubated and examined as mentioned above. Broth culture was then centrifuged in a refrigerated centrifuge at 15,000 r.p.m. for 30 minutes . The obtained pelleted cells were suspended in 10 ml Tris-HCl buffer of pH8 (Tanaka et al., 1987).

Preparation of the hyperimmune serum:

Hyperimmune serum against C.chauvoei. whole cellular antigen prepared by immunization of two New Zealand rabbits with C.chauvoei formalinized whole cells after the method of Traub et al., (1991) with some modification . 50ml. of 24 hours broth culture of C.chauvoei. were mixed with 50 ml of 0.6% formalin and incubated at 37°C for 24 hours. Injection of rabbits was done as follow. In the first day the animals received I/V, 0.5ml.of formalinized culture of C.chauvoei., in day 7,14,19 and 25 the animals received 0.4,0.5,0.8 and 1.0ml. of freshly formalinized C.chauvoei. culture, respectively. On the day 35, blood samples were collected from the immunized rabbits, serum was separated and kept at -20°C till used.

Fractionation of C.chauvoei whole cellular antigen using sodium dodocyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE):

The used protocol was that of the manufac
operator * 50 ul of the sample (whole cel
antigen in lysis buffer Tris-HCl) were mixed
50 ul of sample buffer (2% W/V of SDS: 4
2-mercaptoethanol; 0.05% of bromophenol
and 50% glycerol).

Proteins were separated in a discontinuous
consisting of:

(1) 5% stacking gel: made up of 2.5ml of
Tris-HCl pH at 6.9: 0.2ml of 10% SDS: 3
of 30% polyacrylamide; 13.9 ml disti
water, 100ul of 0.02% ammonium persul
and 10ul of Bio-Rad temed.

(2) 20% running gel: 19.4% Tris-HCl in disti
water pH at 8.9; 0.2ml of 10% SDS; 6.7m
30: polyacrylamide; 2 ml of 50% sucr
5.1ml distilled water; 100ul of 2%
ammonium persulfate and 20ul. Bio-
temed.

Each well of the gel was loaded with 30 ul
samples preparation: 9ul of prestained low ra
Bio-Rad standard was included in each gel.
electrophoresis process was done in the prese
of a special running buffer (15gm glycin,5
SDS in one liter distilled water) for one hou
150 volt. After the running process
completed., the gel was stained with coomas
brilliant blue (1.25gm coomassi brilli
blue;227ml. methanol; 46ml glacial acetic a
and 227ml. distilled water) for 5 minutes, t
destained in destaining solution (2700ml disti
water; one liter methanol and 300 ml. glac

* Manual for protein II slab gel, Bio-Rad, 84-0123984, USA.

acetic acid) several times and finally kept in destaining solution overnight at room temperature.

Western blot (immunoblotting procedure):

For detection of the immunodeterminants of the whole cellular antigen of C.chauvoei. , gels were prepared and electrophoresed as mentioned above but not stained and kept in the running buffer for 15 minutes before transfer. The separated proteins on the gel were transferred onto a nitrocellulose membrane utilizing the Trans-Blot transfer medium (Trans-Blot cell model 1703910, Bio-Rad) in the presence of the transfer buffer (6gm Tris; 288gm glycin; 400ml, methanol and 1600ml. distilled water) for 90 minutes and 100 volt at 4°C. After the transfer process was completed, the membrane was kept in blocking buffer (15gm gelatin and 500ml Tris Buffer Saline solution made up of 4.84 gm Tris:58.48gm sodium chloride and two liter distilled water, pH 7.5). The membrane was then washed three times with Tris Buffer Saline (TBS) solution each for 5 minutes, incubated with the primary antibody (hyperimmune serum) diluted 1:100 in Tween Tris Buffer Saline (TTBS) solution (one liter of TBS solution and 0.5ml, of Tween 20) for one hour at room temperature and washed as before. The membrane was incubated with the secondary antibody (biotynated anti-rabbit IgG) for one hour at room temperature. The membrane was finally transferred to a Petri dish containing 10ml. of alfa chloronaphol substrate (Bio-Rad) for developing of the colour which was stopped by washing the membrane in distilled water.

RESULTS AND DISCUSSION

Effective immunization against clostridial diseases is of extreme importance for control of the clostridial infection. Most black leg disease vaccines are prepared from the formalinized whole cell culture of C.chauvoei. Since the whole bacterial cells has long been used as the immunizing agent, it is of special interst to define the role of any or all proteins constituting the bacterial cell antigen. Polyacrylamide gel electrophoresis has been widely used for separating complex matrix of polypeptides which can be visualized by immunoblotting assay through the interaction with the specific hyperimmune serum prepared against the proteins (Towbin et al., 1979). Using SDS-PAGE, the protein of the whole cellular antigen of C.chauvoei. in the present study (Fig. 1) revealed approximately 8 polypeptide bands of molecular weight ranging from 205 to 18.5 KDa. A major protein band with a molecular weight of 49.5 KDa was detected. Tanura et al. (1985) recorded the presence of one major protein polypeptide of molecular weight of 56.0 KDa and two minor protein bands with a molecular weight of 120 and 200 KDa, respectively, by SDA-PAGE of the whole cellular antigen of C.chauvoei. Moreover, Stevenson and Stonger (1980) identified two major and approximately five minor protein bands in the cellular antigen of C.chauvoei. On the other hand. Traub et al. (1991) recorded more than 15 polypeptides in cell homogenate of C.perfringens using SDA-PAGE. The molecular weight of these bands ranged from 138-14 KDa. The difference in the number of reported bands may be due to the used analysis system and strain variation.

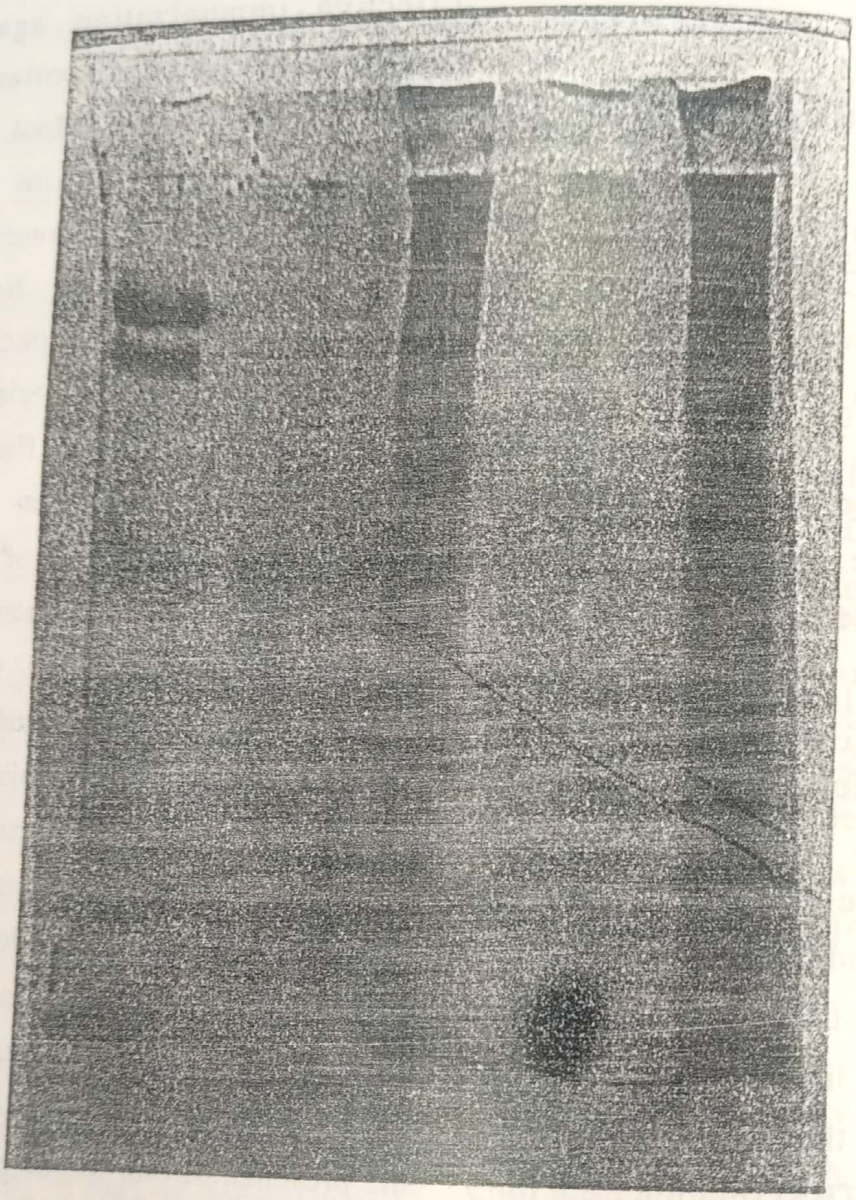

Fig. 1 : SDS-PAGE analysis of *C.chauvoei* whole cellular antigen :
left: low range molecular weight standard.
right & middle : protein profile of the whole cellular antigen of *C.chauvoei*

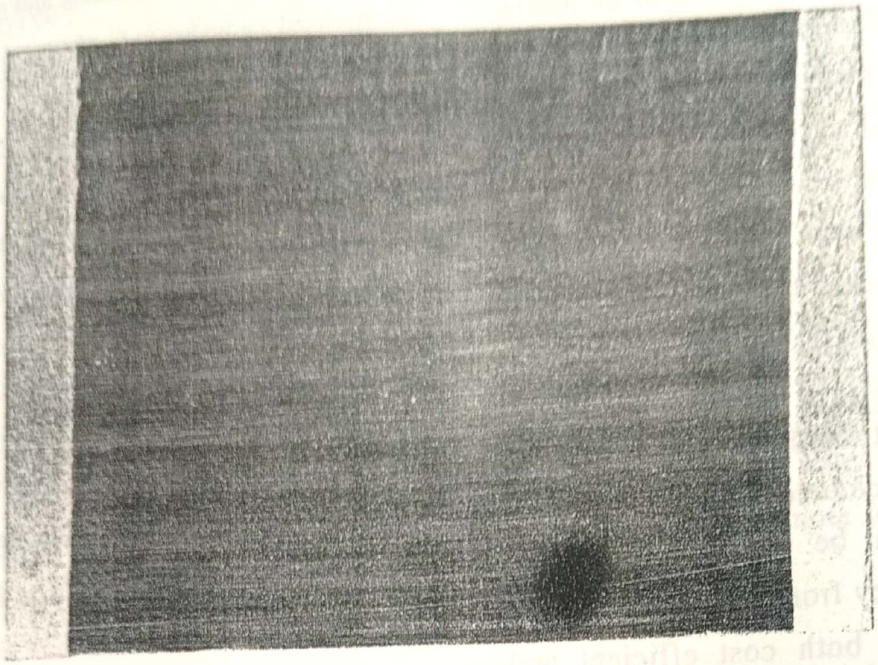

Fig. 2 : Immunoblotting results of the whole cellular antigen of *C.chauvoei* with its specific hyperimmune sera :
Lanes 1&10 : low range molecular weight standard.
Lanes 2-9 : reaction of the whole cellular antigen of *C.chauvoei* with its specific hyperimmune sera .

Results of the immunoblotting procedure (Fig.2) revealed that the hyperimmune serum prepared against whole cellular antigen of C.chauvoei. reacted strongly with the polypeptide bands having molecular weight of approximatelly 205 KDa. In addition another less reactive epitope of approximately 29.5KDa was also observed (Fig.2). Tanaka et al. (1987) found that monoclonal antibody against C.chauvoei reacted strongly with the polypeptide band of molecular weight of 56 KDa and to lesser extent with two or more bands of molecular weight of approximately 200 KDa. Moreover, Traub et al. (1991) showed that, hyperimmune serum prepared against homogenates of C.perfringens reacted against 4-7 polypeptide bands of molecular weight ranging from 138 to 28.8KDa.

From the aforementioned results, it can be concluded that, *C. chauvoei* has its specific epitopes which can be isolated. A future immunizing agent soley from such immunogenic protein would seem both cost efficient and provide more protection to the animals. Further research on such epitopes is needed to determine their roles in conferring complete protection of immunized animals.

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