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Protein Profile of *Clostridium perfringens* Isolated from Animals and Foods of Animal Origin

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Partially purified protein extracts from six randomly selected isolate was subjected to SDS-PAGE to study their protein profile. The isolates exhibited variability in protein profiles in terms of both number of bands and corresponding molecular weights. Five of the preparations were found to share a common protein band with almost similar molecular weights within the range of 33.4 kDa and 34.8 kDa, which indicated production of alpha toxin by the concerned isolates. All these isolates also showed production of DNase and phospholipase C, while haemolysin production was observed in three. In addition three isolates also exhibited a common protein band within the range of 43.1 kDa to 44.6 kDa.

Key words: *Clostridium perfringens*, DNase, haemolysin, phospholipase C, SDS-PAGE.

Clostridium perfringens is an important pathogen responsible for a wide spectrum of human and animal diseases. Limitations of antisera technique and mouse protection test for demonstration of toxins, has led to the development of SDS-PAGE technique to characterise the protein profile of various pathogenic bacteria including *Clostridium perfringens*. Therefore in the present study, 98 (48.27%) *Clostridium perfringens* were isolated from animals and food of animal origin. Protein profile of six isolates randomly selected among these isolates were analysed by SDS-PAGE as per the method of Laemmli[1].

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MATERIALS AND METHODS

The partially purified protein extracts of six randomly selected isolates of *C. perfringens* from clinically affected and apparently healthy animals and birds were subjected to SDS-PAGE for analysis of electrophoretic patterns and to determine the molecular weight of the protein present in the extracts. The isolates selected for the study were recovered from goat faecal sample, muscle tissue of cattle, cattle faecal sample, sheep faecal sample, and yak faecal sample. The test was carried out as per the method of Laemmli using a gel concentration of 10 % per cent. The wells were loaded with 25 ml of samples (40 µg of protein per well). A molecular marker, consisting of five different proteins with molecular weights 116, 66.2, 45.0, 35.0, 25.0, 18.4 and 14.4 kDa was also run for determination of molecular weights of the proteins present in the extracts. The gel was run at 30 mA constant current and 250 volt electricity for 3 hours.

Preparation of samples

Samples for SDS-PAGE were prepared from randomly selected strains of *C. perfringens* isolated from various sources in the present study as per the method described by Amimoto *et al.*[2] with slight modification. Selected isolates were grown by inoculating into the thioglycolate medium (Hi-Media Laboratories Pvt. Ltd., Mumbai) tubes. Inoculated tubes were incubated at 37°C under anaerobic condition for 16 hours (end of logarithmic growth phase). Growths were centrifuged at 16000 rpm for 10 minutes at 4°C and supernatants were collected aseptically. Collected supernatants were partially purified by salt precipitation with ammonium sulphate at 60 per cent saturation level at 4°C for overnight. The samples were centrifuged at 16000 rpm for 10 minutes at 4°C and the supernatants were discarded. The precipitates were dissolved in sufficient volume (half of the volume of ammonium sulphate used) of normal saline solution (NSS). The samples were dialysed extensively in cellophane dialysing sacs (Sigma, USA) against NSS at 4°C until all sulphate ions were removed from the dialysate to NSS that was detected using 1 per cent barium chloride solution. The dialysed preparations were concentrated to half of the original volume by 30 per cent (w/v) polyethylene glycol 6000 (PEG-6000).

The purified protein extracts (25ml) were then mixed in 25ml sample buffer and were boiled at 100°C for 5 minutes and loaded in separate wells. Electrophoresis was done at 30mA for 3 hours in conjugation with marker protein of known molecular weight. The gel was washed and protein bands were stained with 0.25 per cent w/v Coomassie brilliant blue (CBB), destained and fixed in 10 per cent (v/v) acetic acid in water. Analysis of the stained (Coomassie brilliant blue) gel was performed using a gel documentation system (Kodak).

RESULTS AND DISCUSSION

The present study revealed that there was no homogeneity among the isolates in terms of protein profile and their corresponding molecular weights. The extracts prepared from different isolates showed variable number of protein bands with molecular weights ranging from 110.9 kDa to 16.4 kDa. The highest number of protein bands was observed in lane 2 (14) followed by Lane 1 and 4 (10 each), Lane 3 (7) and Lane 5 and 6 (6 each). Details of the protein profile study are shown in Table 1.

Table 1. Protein profiles of various isolates of *C. perfringens* with their molecular weight determined by SDS-PAGE

Band	MolecularWeights of protein bands (Da) in lane numbers						
No.	1	2	3	4	5	6	7
1.	106715.3	89833.9	81393.2	110935.6	100806.8	107559.3	116000
2.	59840.0	73796.6	62262.9	77172.9	58628.6	98274.6	66200
3.	56205.7	69576.3	37386.4	64988.6	39204.5	61051.4	45000
4.	43181.8	63171.4	34056.6	61354.3	33490.6	36477.3	35000
5.	40113.6	56508.6	28773.6	43636.4	26320.8	34245.3	25000
6.	34811.3	44659.1	23186.8	36477.3	22969.2	23984.6	18400
7.	27075.5	42500.0	21228.6	33867.9			14400
8.	23476.9	39659.1		31037.7			
9.	20938.5	36931.8		25943.4			
10.	14929.4	32169.8		23984.6			
11.		25000.0					
12.		21953.8					
13.		17047.1					
14.		16400.0					

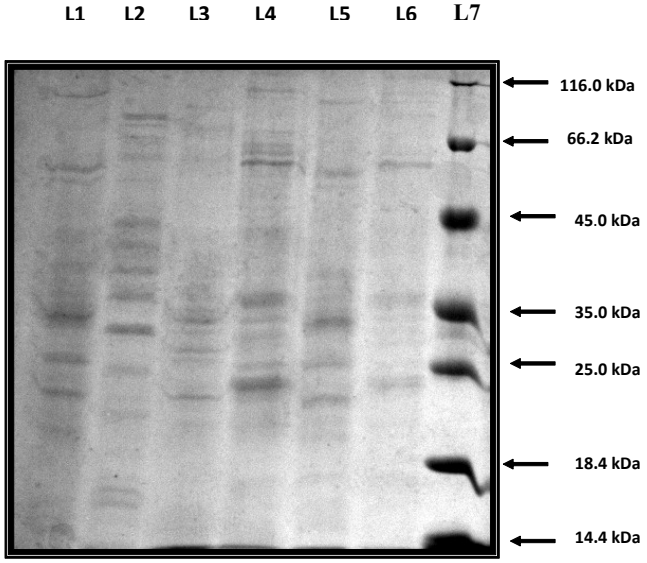
Lane 1 to 6 – Partially purified protein extract from *Clostridium perfringens* from sample from different sources
Lane 1- Goat faecal sample Lane 2- Muscle tissue of cattle Lane 3- Cattle faecal sample
Lane 4- Goat faecal sample Lane 5- Sheep faecal sample Lane 6- Yak faecal sample
Lane 7- 10 kDa Protein marker

The major protein bands exhibited by Lane 2 as analysed by gel documentation system as displayed in Figure 1 were between 89.8 kDa and 16.4 kDa, while the ten protein bands in Lane 1 and 4 were between 110.9 kDa and 14.9 kDa. Molecular weight of the seven protein bands in Lane 3 were between 81.3 kDa and 21.2 kDa. Similarly, molecular weight of the protein bands in Lane 5 and 6 were found to be within the range of 107.5 kDa and 22.9 kDa. Although all the six extracts contained variable number of protein with different molecular weights, five isolates were found to exhibit a common protein band with almost similar molecular weights within the range of 33.4 kDa and 34.8 kDa. In addition, three isolates also exhibited a common protein band within the range of 43.1 kDa to 44.6 kDa.

Mollby and Wadstorm (3) purified phospholipase-C (alpha toxin) from *C. perfringens* and performed SDS-PAGE, which revealed protein bands with molecular weight of 30,000 ± 2000 Da. However, Hirata *et al.* (4) revealed appearance of a single protein band with molecular weights around 43.0 kDa in their study on SDS-PAGE of recombinant phospholipase-C of *C. perfringens*. Cheung and Rood (5) reported that the production of alpha toxin by *C. perfringens* was regulated by a regulatory system comprising of VirR response regulator, encode by *virR* genes. The SDS-PAGE of the purified protein revealed appearance of a single protein band with molecular weight of 33 kDa. Amimoto *et al.* (2) could demonstrate a single protein band with molecular weight of 45 kDa in purified alpha toxin of *C. perfringens*.

It was revealed from the comparative study of the five isolates that shared a common protein band in respect to their virulence properties, *viz*. DNase, haemolysin and phospholipase-C that all of these isolates produced DNase and phospholipase-C, while haemolysin production was observed in three. This result was in agreement with the findings of Takahashi *et al.* (6) who reported that these three activities reside in a single entity.

The present findings suggested that in addition to SDS-PAGE study, tests for phospholipase-C and DNase production in *C. perfringens* isolates might be useful for identification of alpha toxin producing strains. No conclusion could, however, be made in respect to the proteins other than the common protein band detected in the present study. Those bands might represent some minor proteins secreted by the isolates.



L1 to L6 : Purified protein extracts of *Clostridium perfringens* isolates
L7 : Marker protein

Fig. 1. Polyacrylamide gel electrophoretic patterns of purified protein extracts of *Clostridium perfringens* isolates

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