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Polypeptide Mapping of Different Isolates of *Pasteurella multocida* Bovine Origin

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ABSTRACT

The protein analysis of *Pasteurella multocida* organism was performed by sodium dodycyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique using a high pH discontinous buffer system. Six isolates of the *Pasteurella multocida* organism were confirmed by Gram's staining and different biochemical tests. In the protein fractions of purified *Pasteurella multocida* strains, seven polypeptide bands of different molecular weights ranging from 36.31 KDa to 104.71 KDa were observed after gel electrophoresis by SDS-PAGE. Results showed that all the isolates belonged to the same serotype.

***Key Words*:** *Pasteurella multocida*, SDS-PAGE, protein fractioning

INTRODUCTION

Haemorrhagic septicaemia (HS) is an acute, highly fatal septicaemic disease of cattle and buffaloes caused by specific serotypes within the bacterial species of *Pasteurella multocida* (Bain et al., 1982). The annual loss due to this disease in Asia was estimated, one lakh animals. Hudson (1954) worked with 58 strains from buffalo and bovine in India, Pakistan, Thailand and Kenya belonging to type-I. Carter (1955) used indirect haemagglutination test and recognized four serotypes and designated them as A, B, C and D. There were reports in which some of the vaccinated animals succumbed to subsequent infection with *Pasteurella multocida* organisms within short period after vaccination which may be due to either insufficient immunity produced by the bacterin or to infection by strains antigenically different from those used in preparation of vaccine (Sridevi et al., 1999**)**. In order to have a reliable vaccine for use in particular areas, it is important that strain of *Pasteurella multocida* used for the production of vaccine be antigenically similar and immunologically homologous to the strain of organisms prevalent in the field. This study was designed to find out the difference on the basis of protein fractions and the molecular weights of the isolates using SDS-PAGE technique.

MATERIALS AND METHODS

Isolation and Identification

Six isolates of *Pasteurella multocida* organism were isolated from different animals suffering from haemorrhagic septicaemia. After primary isolation, the isolates were confirmed by Gram's staining, different biochemical tests, mice inoculation and reisolation.

Sample Preparation for SDS-Page Technique

The sample was prepared by crude saline extraction of *Pasteurella multocida*. The organism was grown on nutrient agar and colonies of the organism were observed and mixed with normal saline and were frozen then thawed. This process was repeated three

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times in order to lyse the cells. The prepared sample was mixed with 2x-treatment buffer (0.125M Tris-cl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptethanol) and boiled for three minutes. The sample was centrifuged

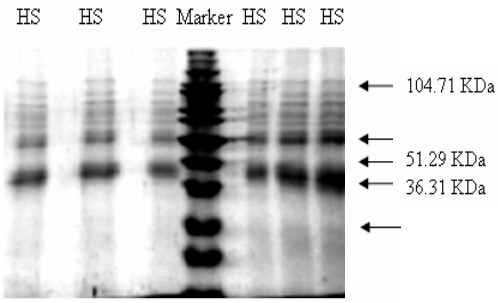

Figure 1 Polypeptide mapping of different isolates of *Pasteurella multocida* bovine origin

at 1500 rpm for 5 minutes. Supernatant was collected and loaded on the gel with standard markers of molecular weights ranging from 10 KDa to 200 KDa (Fermentas®).

***Electrophoresis*:** Sodium dodycyle sulphate polyacrylamide gel electrophoresis technique was performed with discontinous buffer system in vertical fashion as described by Laemmli (1970) using standard markers. Briefly, outer membrane proteins were analyzed using 5% stacking gel and 15% separating gel. The protein concentration in the sample was adjusted at 15-20 µg/µL and an amount of 15µL sample was mixed with 1 µL of bromopenol blue and loaded in gel. Molecular weight marker was also loaded in separate well and electrophoresis was performed by applying 30 mA constant current and 200 V for 45 minutes. For resolving gel 8.8 pH, for stacking gel 6.8 pH and for tank/running buffer 8.3 pH was achieved by adding solutions of NaOH and/or HCl. Separating gel was placed in staining solution over night. Next day staining solution was replaced with de-staining solution and left for 5-6 hrs at constant shaking. Gel was rinsed with distilled water and photographed in white light. A graph was plotted between Rf value and log of molecular weight to determine approximate molecular weight of proteins.

RESULTS AND DISCUSSION

For the analysis of proteins of different isolates of *Pasteurella multocida* organism, SDS-PAGE was run and the gel obtained after analysis was stained with coomassie brilliant blue dye. By this technique different polypeptides of the organism's proteins were observed as shown in Figure 1. The protein profiles of *Pasteurella multocida* whole cell isolates revealed seven bands on SDS-PAGE analysis i.e. 36.31, 51.29, 60.26, 69.18, 77.62, 85.11, and 104.71 KDa, among which 51.29 and 36.31 KDa bands were thick and recognized as major bands. In this study a band of 60.26 KDa was reported that is in agreement with the results of (Sridevi et al., 1999) who described 13 bands of *Pasteurella multocida* whole cell on SDS-PAGE analysis among which 61.27 KDa and 26 KDa bands were major bands. Similarly 5-6 main protein bands with molecular weights of 27, 32, 35, 37 and 44 KDa were observed by (Johnson et al., 1989) when they performed SDS-PAGE of Indonesian vaccine strain, our results also showed 36.31 KDa band as a major band. Studies of Lee et al. (1991) with different strains of *Pasteurella multocida* suggested that there were no serotype specific band patterns with regard to protein and carbohydrate moieties of avian isolates on SDS-PAGE, our studies also revealed similar results in *Pasteurella multocida* organism of bovine origin. Dabo et al. (1997) found eight major outer membrane proteins having two major proteins of 35 KDa and 46 KDa molecular weights by SDS-PAGE analysis of bovine *Pasteurella multocida* isolated from cattle and pig sources. Pati et al. (1996) observed ten polypeptide bands having molecular weights 88 KDa to 25 KDa, among these 44.37 KDa

and 30 KDa bands were considered as major immunogens. Morton et al. (1996) saw eight protein bands on SDS-Page analysis i.e. 94, 84, 53.5, 49, 43, 41, 29.5 and 16 KDa but protein band of molecular weight 43 KDa was present in all serovars. Several workers (Syuto and Matsumoto, 1982; Lugtenberg et al., 1984; Ireland et al., 1991; Lee et al., 1991; Wang and Glisson, 1994) suggested that the number of protein and carbohydrate bands resolved on SDS-PAGE were different from study to study that may be attributed to the type and strain of the antigen.

CONCLUSION

Protein profile of *Pasteurella multocida* organism of bovine origin revealed similar polypeptide bands by SDS-PAGE technique. However, studies should also be carried out to see the comparison between *Pasteurella multocida* of bovine origin and avian origin.

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