

Original Research Article

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Protein Profile Analysis of *Dermatophilus congolensis* Isolates Using Sodium Dodecyl Polyacrylamide Gel Electrophoresis and Western Blotting

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ABSTRACT

Bovine dermatophilosis, also known as cutaneous streptothrichosis in cattle, is a skin infection which causes severe production losses in tropical countries. The present paper reports protein profile analysis of *D. congolensis* isolates from bovines in Kerala and identification of the immunodominant proteins using western blotting. Fifteen isolates of *Dermatophilus congolensis* obtained from dermatitis cases from 14 cattle and one buffalo were included in the study. Protein profile analysis of the isolates using sodium dodecyl polyacrylamide gel electrophoresis revealed variable number of bands with molecular weight in 14 to 130 kDa range. Quantitative as well as qualitative differences were observed in protein patterns obtained from different isolates which indicated the antigenic relatedness and diversity between the isolates. Western blotting of proteins of six isolates using hyper immune sera raised in rabbits recognised protein bands in the 25 to 35 kDa range. Proteins of molecular weights of about 29 kDa was found as major reactor in four of isolates tested, 32 kDa in two isolates, 38 kDa and 40 kDa in one each. The presence of the 29 kDa protein in majority of isolates in the present study indicates a possible role of it in inducing humeral immunity and therefore can be considered as a possible target for the immune response in cattle

Keywords

Dermatophilosis, Bovine, Protein profile, SDS-PAGE, Western blotting

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Introduction

Dermatophilosis is an infectious bacterial skin disease of domestic, aquatic and wild animals caused by *D. congolensis*. The disease is mostly reported from tropical and subtropical countries. It is also known as rain rot, rain scald, lumpy wool disease, strawberry foot rot, and cutaneous streptothrichosis. Dermatophilosis has been reported as one of the four major bacterial diseases affecting cattle and other animals in the tropical and subtropical regions by the Food and

Agricultural organization. It has been reported from different states in India, including Kerala (Pal, 1995; Tresamol *et al.*, 2015a). The disease is characterised by exudative epidermatitis with matting of hairs and thick scab formation. Variable clinical signs and differences in the distribution of lesions were reported in clinical cases of bovine dermatophilosis (Koney, 1996; Tresamol *et al.*, 2015b). Significant genetic diversity among *D. congolensis* isolates from cattle, sheep, horses, goat, marsupial and chelonids by genetic analysis was demonstrated (Trott *et*

al., 1995). The *D. congolensis* isolates were differentiated by establishing the protein profiles by the use of sodium dodecyl polyacrylamide gel electrophoresis (Masters *et al.*, 1995; Makinde and Gyles, 1999). The present paper involves protein profile analysis of *D. congolensis* isolates from lower leg dermatitis cases in cattle in Kerala and identification of the immunodominant proteins using western blotting.

Materials and Methods

Cultural isolation

Skin swabs and scabs were collected from clinical cases of wide spread lower leg dermatitis lesions of cows and buffaloes and were subjected to cultural examination as per Haalstra (1965).

Preparation of whole cell proteins

Cultures of the 15 representative isolates of *D. congolensis* including 14 isolates from cattle and one from buffalo were inoculated in to brain heart infusion broth and incubated at 37°C overnight. One millilitre of each of the samples was transferred to eppendorf tubes and centrifuged at 10,000 x g for five minutes. The pellets obtained were then suspended in Phosphate buffer saline (PBS, pH 7.2) and centrifuged at 10,000 x g for five minutes. The washing procedure was repeated for two more times. The pelleted and washed cells were then resuspended in sample preparation buffer, boiled for 10 min. and used as whole cell proteins of *D. congolensis*.

SDS-PAGE gel electrophoresis

The protein profile analysis of 15 isolates was carried out using one dimensional SDS-PAGE as per method described by Laemmli (1970), in a vertical electrophoresis apparatus (Hofer, USA) with minor modifications.

Clean Glass plates were set in the gel moulding tray of electrophoresis apparatus. Four millilitres of 12 per cent resolving acrylamide gel solution was poured and one millilitre of water was layered over the gel and allowed to polymerize. Removed the water and poured 1.5 ml of stacking gel (five per cent) over the resolving gel. A comb of suitable size was inserted and the apparatus was kept undisturbed for complete polymerization to occur. Removed the comb after complete polymerization and the gel was mounted on to the electrophoresis chamber. Tris - glycine buffer was used for filling the buffer reservoirs. The samples (20 µl) were loaded in the wells and a standard protein medium range molecular weight marker was also loaded in one well. The electrophoresis was carried out at a constant voltage of 50V till the dye front crossed the stacking gel. Subsequently the voltage was increased to 100V till the dye front reached end of the gel. The power was disconnected and the gel was removed from the glass plate. The stacking gel was snipped off and the resolving gel was subjected to the Coomassie brilliant blue staining for one hour followed by destaining for three to four hours with three to four changes of destaining solution at intervals till the background became clear. Finally the gel was transferred to distilled water and viewed in white light and photographed. Apparent molecular weights were determined by comparing with molecular weight of the reference protein standards.

Western blotting

The proteins of six isolates fractionated in the SDS-PAGE gel were transferred on to a Nitrocellulose membrane (NCM) as per Towbin *et al.*, (1979) with minor modifications.

After fractionating the proteins by SDS-PAGE, the gel sandwich was disassembled

and kept in transfer buffer for five to ten minutes. Transfer membrane, prepared by cutting NCM to the same size as that of gel, was then placed in to distilled water slowly at 45° angle. Once it was fully wet, equilibrated for 15 minutes in transfer buffer. Eighteen Whatmann No.1 filter paper sheets were cut to gel size and soaked in transfer buffer. A microtitre plate was placed in the centre of a large Petri plate and it was filled with blot buffer to a level just below the microtitre plate. A large glass plate was placed above the microtitre plate and a large Whatmann No.1 filter paper of size larger than the gel wetted with transfer buffer was placed above the glass plate with ends immersed in blot buffer. Nine gel sized equilibrated filter papers were stacked above this large filter paper and NCM was placed above this stack. The gel portion soaked in transfer membrane was placed over this NCM and nine equilibrated filter papers were stacked over this assembly. A glass rod was then rolled over the assembly to ensure that there were no air bubbles trapped between the gel and NCM. On the top of this, a glass plate and a sufficient weight were kept. This assembly was left overnight at 4°C to ensure complete transfer.

The transfer unit was disassembled and the membrane was removed after marking the orientation and the gel was subjected to staining and destaining as described previously to verify the transfer efficiency. The membrane was placed in five millilitre of blocking buffer and incubated at 37°C for 2 hours, followed by washing twice with TTBS for 10 minutes each. Primary antibody *i.e.* hyper immune serum raised against *D. congolensis* in rabbit was diluted in blocking buffer (1:100) was added to the membrane and incubated for 45 minutes at 37°C with constant agitation. The membrane was washed four times for 15 minutes each with sufficient amount (100 to 200 ml) of TTBS.

The membrane was then incubated at 37°C in diluted horse radish peroxidase conjugate (1:2500, in blocking buffer) for one hour with intermittent shaking. The membrane washing procedure was repeated as before and blots were developed by keeping the membrane in to chromogenic visualization solution at room temperature with gentle rocking until the colour was developed. The reaction was terminated by washing the membrane with distilled water. Membrane was air dried and photographed.

Results and Discussion

Culture of skin scabs in sheep blood agar yielded typical greyish beta haemolytic adherent colonies which were further confirmed as *D. congolensis* by morphological appearance and biochemical reactions. Protein profile analysis of 14 isolates from cattle and one isolate from buffalo by SDS PAGE revealed variable number of bands with variable intensity of colour. Molecular weight of the protein fractions ranged from 14 to 130 kDa in all the isolates. Quantitative and qualitative differences were observed in protein patterns of different isolates. Two bands of approximate molecular weights of 23 and 57 KDa were observed in common with majority of isolates. These common bands indicated the relatedness between the isolates. Similar observations were also reported by Shaibu *et al.*, (2011) with common bands at about 20 and 62 KDa size in *D. congolensis* isolates from cattle, sheep and goats. Protein profile analysis of ovine isolates of *D. congolensis* by Gogolewski *et al.*, (1992) also revealed common bands at 30 and 76 KDa. Bands corresponding to the proteins of molecular weight 30 and 97 KDa were observed by Kruger *et al.*, (1998) among isolates from horses. Similar observations were also made by other workers (Makinde and Gyles, 1999; Shaibu and Adetosoye, 2008).

Plate.1 Protein Profile of *D.congolensis* on SDS-PAGE

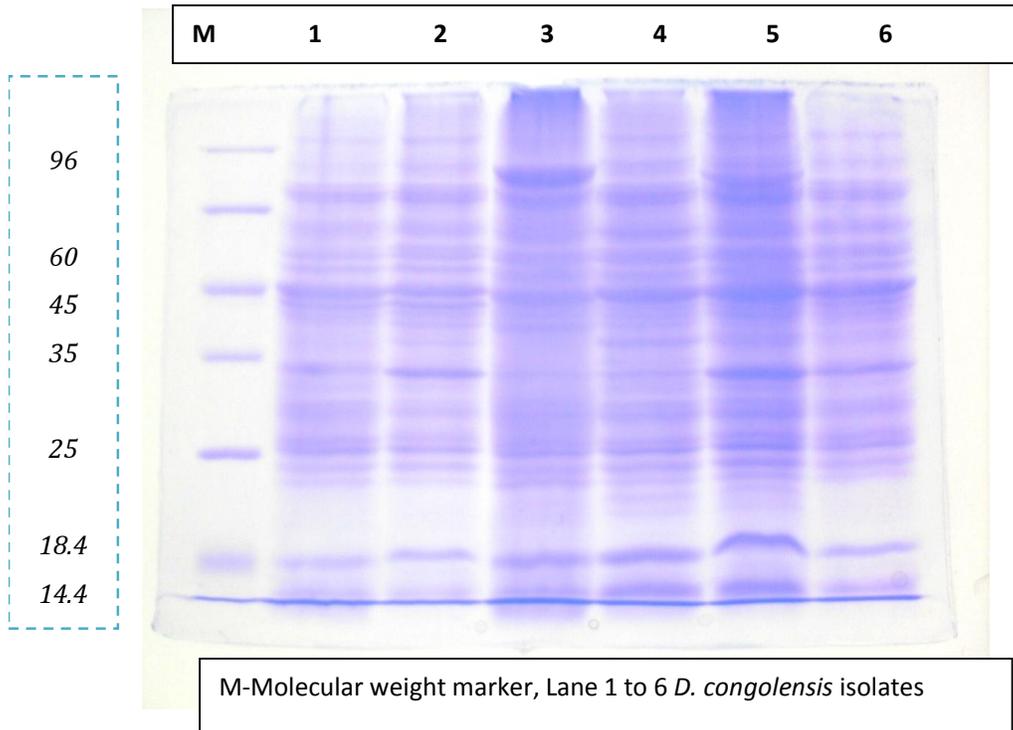
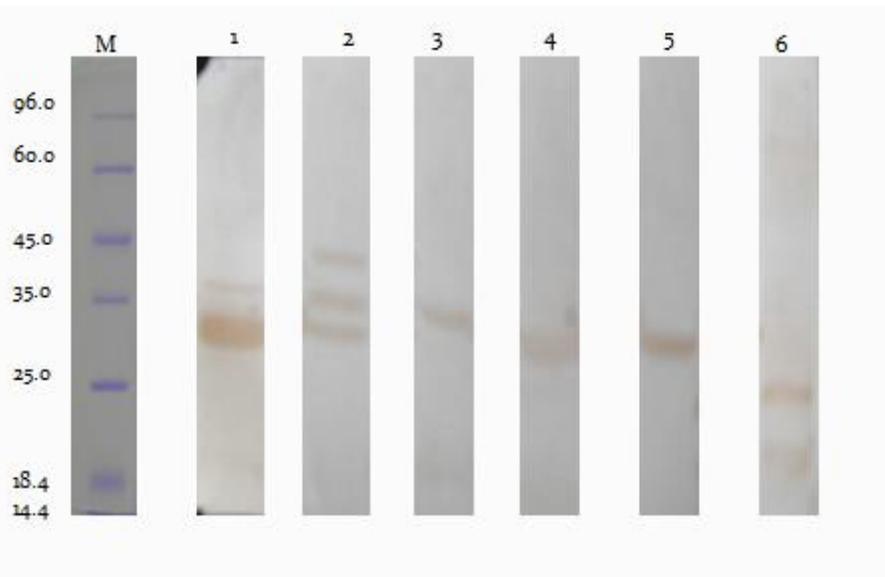


Plate.2 Western blotting of *D. congolensis* isolates



M-Molecular weight marker, Lane 1 to 6 *D. congolensis* isolates

There were also other bands that were common in some of the isolates, which are indicative of relatedness among that isolates.

The isolate from buffalo showed similar protein profile pattern with three of isolates from cattle. There were variations in the

presence of the other major and minor protein bands between the isolates. Protein bands of molecular weight of about 14 KDa was present in 10 out of 15 isolates, bands at 32 KDa in nine isolates, bands at 18 KDa in seven isolates, bands at 85Kda in six isolates and bands at 110Kda in six isolates.

The variations in the protein profile pattern with differences in the molecular weights by different workers might be due to difference in the methods of measurements and calculations as suggested by Shaibu *et al.*, (2011). Many factors such as pH, ionic properties of water, source and age of the reagents used may influence the relative mobility of the proteins in the gel. The protein patterns observed in isolates in the present study indicated antigenic similarities and differences among the isolates. Similar reports were also made by Makinde and Gyles (1999), Ellis *et al.*, (1993) and Kruger *et al.*, (1993) and they could not associate the variations with either geographic, climatic or host factors.

Western blotting of proteins of six isolates using hyper immune sera raised in rabbits recognised protein bands in the 25 to 35 KDa range. Proteins of molecular weights of about 29 KDa was found as major reactor in four isolates tested, 32 KDa in two isolates, 38 KDa and 40 KDa in one each. Isolate 6 had different reactors of approximate molecular weights 18, 25 and 85 KDa. Gogolewski *et al.*, (1992) identified two immunodominant proteins of molecular masses of about 76 and 31 KDa in western blots of *D. congolensis* isolates from sheep.

Majority of the protein bands were not visualised in immunoblots. This might be due poor immunogenicity of these proteins. Difference in the immunodominant proteins between isolates was suggested as the reason for failure of immunisation against

dermatophilosis in the field by Makinde and Gyles (1999). The presence of the 29 KDa protein in majority of isolates in the present study indicates a possible role of this protein in inducing humoral immunity and therefore can be considered as a possible target for immunisation in cattle. However, a detailed study employing more number of isolates is needed to confirm the immunogenicity of proteins.

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