# COMPARISON OF CRUDE PROTEIN PROFILES OF ISOLATES OF *Dermatophilus congolensis* FROM CATTLE BY SDS-PAGE

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## SUMMARY

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis was used in the analysis of protein profiles, for the characterization of eight isolates of *Dermatophilus congolensis* from clinically infected cattle. All the eight isolates had a band at the 21KDa protein level. Seven isolates had a band at the 70kDa protein level. This is an indication that all the isolates are related and could belong to the same species. Five of the isolates were more closely related to one another, than the others, by having three similar proteins bands at the 72, 70, and 66KDa. Level. Sodium dodecyl sulphate poly acrylamide gel electrophoresis is suggested as a routine adjunct method to the present method of characterization and identification of *Dermatophilus congolensis*.**

## INTRODUCTION

Dermatophilosis is a skin disease caused by the Actinomycete Dermatophilus congolensis, a strongly Gram-positive organism with a multiphasic life cycle (4, 19). The disease is economically important in cattle in the tropical regions and in sheep in high rainfall areas (12). The economic importance of the disease is estimated in losses of millions of US dollars, due to loss of productivity in terms of work by infected draft oxen; decrease meat and milk production; a failure of reproduction in cows with severe vulva infection and stud bulls with severe leg lesions making them unable to mount (13); death, due to starvation of calves of dams with infected udders (9); the loss of hides and skins; the cost of chemotherapy and chemoprophylaxis; the cost in time and effort in the control of the disease; as well as losses due to the culling and death of infected animals.

Beaton (2), reported that a wide variety of animals are affected by the disease, ranging from domestic to wild and aquatic animals. The affected domestic animals include cattle, sheep, goats and horses. Because of the wide host range the organism was classified according to the animal species it affected (3, 6, 18). However, Autwick (1) reported that all the classifications belonged to the same organism in the order Actinomycetales in the family Dermatophilaceae with a single genus Dermatophilus and under the name Dermatophilus congolensis (16).

Biochemically, the isolates of Dermatophilus congolensis from different animal species have been found to have slight variations (10, 15). Due to the wide host range, geographical distribution of the disease and the nature of the organism, it is evident that clinical, cultural morphological and biochemical methods are not enough to characterize Dermatophilus congolensis into distinct strains or species.

The aim of this investigation is to introduce other techniques such as Sodium dodecyl Poly Acryl amide Gel Electrophoresis (SDS-PAGE) to analyze the protein patterns of isolates of Dermatophilus congolensis from cattle for identification of variations within the isolates for possible characterization into distinct species.

## MATERIALS AND METHODS

Dermatophilus congolensis Isolates

Skin scabs from clinically infected cattle with Dermatophilosis were collected in clean Bijou bottles, labeled and brought to the laboratory, and processed for the isolation of Dermatophilus

congolensis.

Culture conditions

The organisms (Dermatophilus congolensis), were isolated from the skin scabs collected as previously described (5), with slight modification. Briefly the samples were pulverized and suspended in distilled water in Bijou bottles and incubated at 37°C for 45 minutes, under 10 % CO2. These were then brought out and a loopfull from each suspension was then plated out on 10% blood agar and incubated at 37°C for 48-72 hours under 10% CO2.

Preparation of whole cell proteins

Cultures of the isolates on blood agar plates were harvested in eppendorf tubes and washed three times in phosphate buffered saline, pH 7.2, by centrifugation at 10,000g for five minutes.

The washed cells were suspended in sample treatment buffer (double working strength of sample buffer) containing 125mM Tis-HCl, 4%SDS, 2% Mercaptoethanol, 20% w/v glycerol and then boiled for10 minutes.

The suspension was then centrifuged at 14,000 g for five minutes and the supernatant transferred to fresh eppendorf tubes. Sample buffer (8) containing 2%SDS, 4%Mercaptoethanol, 10% W/V glycerol, 0.1% Bromophenol blue dissolved in 0.625mM Tris HCl pH 6.8 was added to the supernatant and used as Dermatophilus congolensis whole cell proteins.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

A discontinuous SDS-PAGE was performed with 4% stacking gel and a 12% separating gel. The various extracts were solubilized by boiling for 5 minutes at 100 0C in Laemmli solution(containing 2%SDS, 4%Mercaptoethanol, 10 %W/V glycerol, 0.1% Bromophenol blue dissolved in 0.625mM Tris HCl pH 6.8).

The samples were loaded at 20ul per lane and separated in 0.75mm thick gel slabs in the mini protein 11Dual slab gel (Biorad laboratories, Rockville, NY). Electrophoresis was carried out at a constant voltage of 200 volts for 45 minutes until the tracking dye was approximately 1cm from the bottom of the gel.

Pre-stained molecular weight marker. (Biorad laboratories, Rockville, NY) containing lysozyme 14400 Daltons, soybean, trypsin inhibitor 21,500 Daltons, carbonic anhydrase31,000 Daltons, ovalbumin 45,000 Daltons, bovine serum albumin BSA 66,200 Daltons, Phosphorylase B92,500 Daltons, B. Galactosidase 116,200 Daltons and myosin 2000,000 Daltons were included as reference proteins. Bands were visualized by fixing gels and staining for 1-2 hours in a solution of 0.2% coomassie blue R. 250 (Biorad laboratories, Rockville, NY) in 50% methanol and 10% acetic acid.

Apparent molecular weights were calculated by comparison with known molecular weight standards.

Apparent molecular weight was determined by plotting a graph of the Rf (distance migrated by known protein/distance from origin to the end of the gel), against the logarithm of their corresponding molecular weights. The point on the graph which corresponded to the value for the protein of unknown molecular weight was located and the value which corresponds to this on the logarithmic scale was taken as the value of the estimated molecular weight of the protein.

## RESULTS

All the samples, including the molecular weight standard showed protein bands on the sodium dodecyl sulphate polyacrylamide electrophoresis gel that can be visualized. This is an evidence that the proteins in the samples (*D. congolensis)* were separated by the electrophoresis. However, differences in the protein bands from one sample to another were found.

Figure1 shows the photograph of eight *D. congolensis* isolates ran on a gel. The isolates are designated *D. congolensis* A, B, C, D, E, F, G and H. The *D. congolensis* isolates were loaded thus; *D. congolensis* isolate A in lane 1, *D. congolensis* isolate B in lane 2, *D. congolensis* isolate C in lane 3, *D. congolensis* isolate D in lane 4, *D. congolensis* isolate E in lane 5, *D. congolensis* isolate F in lane 6, *D. congolensis* isolate G in lane 7 and *D. congolensis* isolate H in lane 8.Three prominent bands of approximate molecular weight of 72kDa, 70kda and 66kDa were visualized. The protein bands were labeled (a) for 72kDa, (b) for 70kDa (c) for 66kDa and (d) for the faint protein band. Three prominent bands

of approximate molecular weight between 72kda, 70kda and 66kda can be visualized from D. congolensis isolates A, B, D, E, & F in lanes 1, 2, 4, 5 & 6 respectively and one faint band at the lower level corresponding to approximately 21kda. Isolates C, G, & H in lanes 3, 7, & 8 respectively did not show very prominent bands. Isolate C has three bands at the upper level with the highest corresponding to approximately 70kDa. It also has one faint band at the 21kda level. Isolate G shows only one unclear band at the 21kda level, isolate H shows two bands at the 66 and 70kda level and one at the 21kDa level.

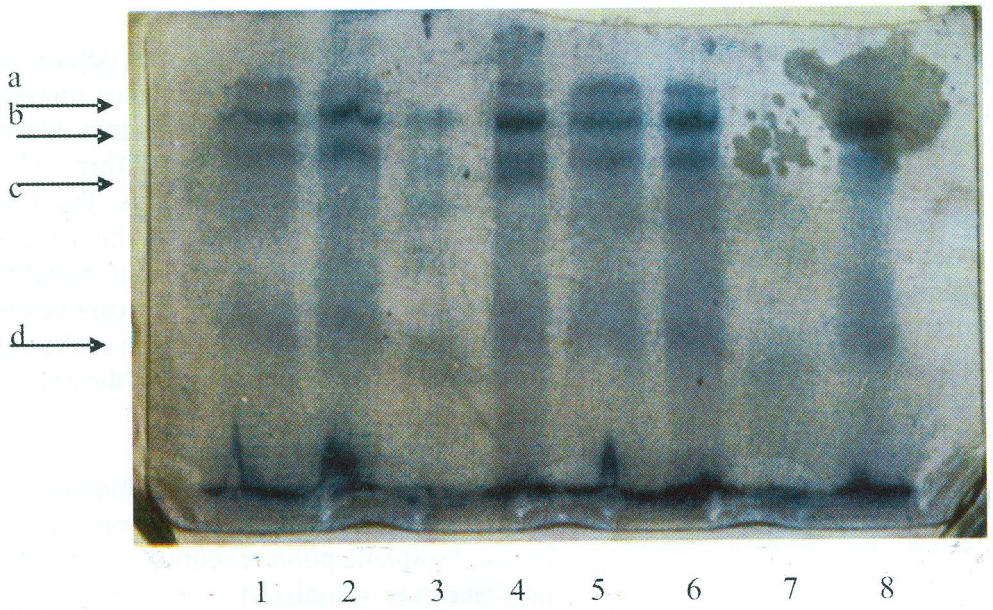


**Fig.1. Shows Eight *D. congolensis* isolates designated A to H**
***Lane 1: isolate A, lane 2: isolate B, lane 3: isolate C, lane 4: isolate D, lane 5: isolate E, lane 6: isolate F, lane 7: isolate G and lane 8: isolate H.***
***Prominent protein bands are labeled (a), (b), (c) and (d).***

## DISCUSSION

Sodium dodecyl sulphate polyacrylamide gel electrophoresis of whole cell protein pattern profiles of microorganisms may be utilized as an adjunct for their characterization and identification. All the eight *D. congolensis* isolates used for this study showed distinct protein profiles.

All the isolates have a common band of approximately 70kDa molecular weight, except isolate 7. All the isolates also shared a faint band at the 21kDa level. This is an indication of the genetic relatedness of the isolates as it has been shown that, proteins are genetically-directed and their patterns tend to express genetic identity of a particular organism as well as its relatedness to other micro-organisms. Five of the isolates have an extra band of about 72kDa, which could indicate their closer relation as members of the same species. Two isolates share the 70kda band and may be grouped together. Isolate H seems different from the remaining isolates in that it only shares the faint band at 21kDa level.

From the banding patterns observed, five of the eight *D. congolensis* isolates appear to belong to one strain, while two of the isolates can be grouped together as one strain. The remaining one isolate could be regarded as a separate strain.

The heaviness and faintness of protein bands visualized could be explained as the proteins exhibited at the time of harvest of the organisms, or the quantity of the particular peptide extracted during protein preparation, or better still the quantity of protein loaded during electrophoresis (11).

Three protein bands 70kDa, 66kDa and the faint 21kDa seem to be common to all the *Dermatophilus congolensis* isolates, and these could be used as standard bands for the identification of *Dermatophilus congolensis* isolates. These observations agree with that of Makinde and Gyles (11).

Strain differentiation is possible by comparison of protein profiles (14, 17), as the authors used similar technique to identify species of the family Bacteriaceae. Similarly polyacrylamide gel electrophoresis has been used to enhance the characterization of numerous microorganisms. Jackman (7) also used protein patterns in bacteria taxonomy. Strom *et al*, (17) suggested that if polyacrylamide gel electrophoresis is to be used as an adjunct for identification, it would be necessary to use extract of known standards or controls in each electrophoretic run. These standards could be in the form of freeze-dried extracts.

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