



Comparative analysis of whole cell proteins of *Rhodococcus equi* isolates using SDS-page

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Abstract: In the present study, the antigenic profile of four strains of *R. equi* isolated from nasal swabs of foals suffering from respiratory problems from different locations in Haryana was studied using SDS-PAGE in comparison to a standard strain collected from MTCC. This revealed that strain NS25 had 04 additional bands and 02 bands missing in comparison to standard strain. Strain NS44 had 07 additional and 02 missing bands. Strain NS48 had 07 additional and 07 missing bands whereas strain NS79 revealed 06 additional and 03 missing bands in comparison to standard strain. This showed that all the strains originating from varied locations were exhibiting different protein banding patterns thus varied on molecular epidemiological basis.

Keywords: *Rhodococcus equi*, foals, antigenic profile, pneumonia, SDS-PAGE

Introduction

The major causes of pneumonia in foals are bacterial in nature, among which *Rhodococcus equi* is the most prominent. *R. equi* is a nonmotile, non-spore forming pleomorphic gram-positive coccobacillus and a facultative intracellular pathogen of macrophages. *R. equi* previously was described as *Corynebacterium equi* and currently is grouped with aerobic actinomycetes. *R. equi* pneumonia is typically manifested as a chronic suppurative bronchopneumonia with extensive abscessation and associated suppurative lymphadenitis (1). *R. equi* infection has also been described in cattle, sheep, goat etc., but disease in these species is rare, with lesions confined to lymph node (2, 3 & 4). The disease is having zoonotic potential in grazing animals like horses, goats etc. The key to the pathogenesis of *R. equi* is related to the ability of the organism to survive and replicate within alveolar macrophages by inhibiting phagosome-lysosome fusion after phagocytosis (5 & 6). It is an opportunistic pathogen in patients who are immune-compromised, especially those with AIDS and has become a disease of increasing significance in human medicine (7 & 8).

Molecular techniques are major tools for the characterization of bacteria from biological substances. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is frequently used for the

characterization of bacterial macromolecules and it has the advantage of being fairly simple and rapid to perform.

Materials and Methods

Isolation, purification & maintenance of strains
Different strains of *R. equi* (NS25, NS44, NS48, NS79) isolated from nasal swab of foals & one standard strain from MTCC were first cultured on nutrient agar media under aseptic conditions to obtain pure colonies. Further supplemented on Brain Heart Infusion (BHI, Hi Media Labs Pvt. Ltd.) broth, an enriched non-selective medium intended for the cultivation of most anaerobic bacteria and other fastidious microorganisms at 37°C & pH 7.4 with shaking (200 rpm) for 24 hours. Gram staining was performed to check out contamination.

Disruption of cells for whole cell protein & protein estimation

The cultures were centrifuged at 10,000 rpm at 4°C for 30 min and pellet was washed with Phosphate buffer saline (PBS). The pellets were suspended in PBS and sonicated at 3500 rpm for 3 min. at 4°C. Finally stored at -20°C for further use. Protein estimation was done by Lowry's method (9).

SDS-PAGE

The solubilized whole cell proteins were subjected to SDS-PAGE using 12% (w/v) separating

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gel and 5% (w/v) stacking gel. 12% running gel (1.5M Tris-HCl, pH 8.9, 10% SDS, 10% acryl amide + bisacrylamide) & 5% stacking gel (0.5 M Tris-HCl pH 6.9, 10% SDS, 30% acryl bis) were prepared and polymerized by adding 10 µl TEMED & 30 µl APS to the running gel and 5 µl TEMED & 15 µl APS to the stacking gel solution respectively. A marker of known molecular weight (Thermo Fisher Scientific) was also loaded (20µl) along with the samples. Electrophoresis was performed at 100v and 80mA for one and a half hour until the marker had reached the bottom of the gel.

Staining and destaining of gel

Immediately at the end of electrophoresis run, the gel mould was removed and the glass plates of the mould were pried apart with a spatula. Slab gel was stained overnight in 500 ml of staining solution (Coomassie brilliant blue stain). Next morning the staining solution was poured off and replaced with 500 ml destaining solution (7.5% acetic acid). Destaining was done until the background was clear.

Determination of molecular weight of proteins

Molecular weights of whole cell proteins were analyzed by SDS-PAGE by using a high molecular weight standard marker. The molecular weight of marker ranged between 66 kDa to 14 kDa. The marker had 07 proteins bands. The distance traveled by bands of the marker was measured. A standard graph was plotted by taking into account the distance traveled by the bands of marker (in mm) along x-axis and molecular weight (in kDa) of the proteins present in the marker along y-axis. The distance covered by each band of the samples was calculated and with the help of standard graph, molecular weights of proteins present in the sample were determined.

Results and Discussion

Characterization of Bacteria

Pinkish-orange colonies of *R. equi* were observed when grown on nutrient agar plates and thread like growth settled at the bottom when grown in BHI broth in flask.

Staining

Blue rod or cocci shaped cells were observed along with Chinese letter arrangement when Gram staining was done revealing that it was gram positive as shown in Fig. 1.

Protein estimation

On the basis of protein content, standard strain contained maximum protein with conc. 5.5 mg/ml, NS25 having the highest among the four strains (5.0 mg/ml) and NS48 having the lowest as 1.7 mg/ml as shown in table 1.

Protein profiles of the organisms studied in SDS-PAGE

Strain NS25 had 04 additional bands and 02 bands missing in comparison to standard strain. Strain NS44 had 07 additional and 02 missing bands. Strain NS48 had 07 additional and 07 missing bands whereas strain NS79 revealed 06 additional and 03 missing bands in comparison to standard strain as shown in table no. 3 & 4. Protein bands at 66 kDa & 45 kDa were present in all the five strains, 33.5 kDa in strains NS25 & NS79, 29 kDa in strains NS25, NS44, NS48, Standard, 24 kDa in NS48, 20.1 kDa in NS48 & NS79 and 14.2 kDa in NS25, NS44 & Standard.

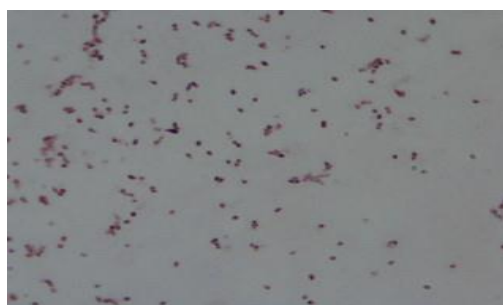


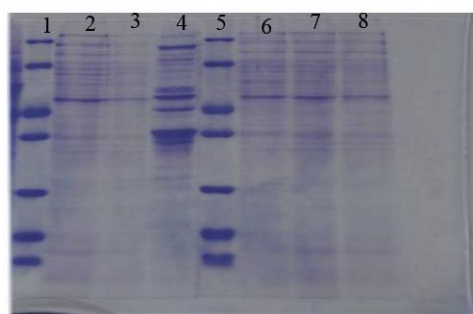
Fig.1: Gram stained *Rhodococcus equi* cells

Table 1. Concentration of protein estimated

S.No.	Sample	Concentration (mg/ml)
1.	NS 25	5.0
2.	NS44	3.6
3.	NS48	1.7
4.	NS79	4.5
5.	Std	5.5

Table 2: Molecular weights of marker proteins

S.No.	Code	Protein	Mol wt. (Da)
1.	A-7517	ALBUMIN, Bovine Plasma	66,000
2.	A-7642	ALBUMIN, Egg (Ovalbumin)	45,000
3.	G-5262	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE	33,500
4.	C-2273	CARBONIC ANHYDRASE	29,000
5.	T-9011	TRYPSINOGEN	24,000
6.	T-9767	TRYPSIN INHIBITOR	20,100
7.	L-6385	oC- LACTALBUMIN	14,200



Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8
Marker	Strain 25	Strain 44	Strain 48	Marker	Strain 79	Std	Std

Fig.2: SDS-PAGE Gel showing location of bands of proteins in different samples

Table 3: Location of the bands observed in the gel is shown below in the table: (in mm)

S.No.	Marker	25	44	48	79	Std
1.	-	3.5	3.5	3.5	3.5	3.5
2.	5.0	5.0	5.0	5.0	5.0	5.0
3.	-	5.5	5.5	5.5	5.5	5.5
4.	-	7.0	-	7.0	7.0	7.0
5.	-	8.0	8.0	-	8.0	8.0
6.	-	-	-	9.0	9.0	9.0
7.	10	10	10	10	10	10
8.	-	-	-	-	-	10.5
9.	-	11	11	11	11	-
10.	-	12	12	-	12	12
11.	-	-	-	13	-	-
12.	-	14	-	-	14	14
13.	-	-	15	15	-	-
14.	-	18	-	-	18	18
15.	-	19	19	19	-	19
16.	-	20	-	20	-	-
17.	22	22	-	-	22	-
18.	-	25	-	25	-	-
19.	27	27	27	27	-	27
20.	-	29	-	-	-	29
21.	-	30	-	-	-	30
22.	-	-	-	34	-	-
23.	39	-	-	39	-	-
24.	49	-	-	49	49	-
25.	55	55	55	-	-	55

Conclusions

Successful approach for the whole cell peptide analysis of four bacterial isolates in comparison to a standard strain has been attempted through SDS-PAGE. Different strains originating from different locations were exhibiting different protein banding patterns thus showing that they varied on molecular epidemiological basis.

SDS-PAGE is an appropriate technique for the identification at species level. Further studies can devise methods for better control of diseases in foals caused by this bacterium. Construction of a vaccine by antigenic peptides and designing of a vaccine by computational biology can be achieved if the peptides of this bacterial species are isolated.

Table 4: Molecular weights of different proteins of different *R. equi* samples were calculated using logarithmic graph: (kDa)

S.No.	Marker	25	44	48	79	Std
1.	-	71,000	71,000	71,000	71,000	71,000
2.	66,000	66,000	66,000	66,000	66,000	66,000
3.	-	64,000	64,000	64,000	64,000	64,000
4.	-	52,000	-	52,000	52,000	52,000
5.	-	48,500	48,500	-	48,500	48,500
6.	-	-	-	46,750	46,750	46,750
7.	45,000	45,000	45,000	45,000	45,000	45,000
8.	-	-	-	-	-	43,500
9.	-	43,000	43,000	43,000	43,000	-
10.	-	41,500	41,500	-	41,500	41,500
11.	-	-	-	40,000	-	-
12.	-	39,000	-	-	39,000	39,000
13.	-	-	38,000	38,000	-	-
14.	-	36,000	-	-	36,000	36,000
15.	-	34,500	34,500	34,500	-	34,500
16.	-	34,000	-	34,000	-	-
17.	33,500	33,500	-	-	33,500	-
18.	-	30,000	-	30,000	-	-
19.	29,000	29,000	29,000	29,000	-	29,000
20.	-	28,000	-	-	-	28,000
21.	-	27,500	-	-	-	27,500
22.	-	-	-	26,000	-	-
23.	24,000	-	-	24,000	-	-
24.	20,100	-	-	20,100	20,100	-
25.	14,200	14,200	14,200	-	-	14,200

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