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COMPARATIVE EVALUATION OF FAECAL COMMUNITY DNA ISOLATION METHODS IN CAMELS

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ABSTRACT

In the present study, a total of 4 fresh and 4 frozen camel faecal samples were subjected to DNA isolation and evaluated from three different published protocols and one commercial kit protocol - QIAamp®DNA stool mini kit (Qiagen, GmbH, Hilden, Germany). Camel faecal DNA isolation using QIAamp®DNA stool mini kit was found the best method in terms of its efficiency, easiness and rapidity of the method.

Key words: Camel, DNA isolation, faeces

Culture- independent methods are now widely used to explore the microbial diversity, where the presence of viable DNA is also not required and further providing a more complete analysis of microbial community structure (McOrist *et al,* 2002; Ariefdjohan *et al,* 2010). On the spot DNA isolation methods are often used now to avoid the disadvantages of culturing the microbes (Espy *et al,* 2006). Methodological comparisons of various protocols have demonstrated that methods of DNA extraction are critical parameter in analysing its microbial diversity (Frostegard *et al,* 1999; Yang *et al,* 2008).The methods used in this paper are already established protocols and are widely applied to extract DNA from faeces of various ruminants. To find a suitable community DNA extraction method for camel faeces, 4 different published protocols were evaluated and compared in terms of their rapidity, easiness, efficiency and storage conditions.

Materials and Methods

Fresh faecal samples were collected in sterile faecal sampling tubes from 8 healthy camels present in and around Bikaner city. Community DNA extraction from 4 fresh faecal samples was done immediately after the collection of samples and 4 samples were kept at -20°C for one month before extraction of the DNA. The faecal samples were processed separately for individual protocol as described below. The community faecal DNA extraction protocols were repeated for all the samples.The 4 different protocols are briefed below:

1) Protocol 1 (Yu and Morrison, 2004)- This protocol is a modified phenol free repeat bead beating method [referred to as repeated bead beating plus column (RBB+C) method]. A 0.25 gm faecal sample was beated in the presence of 1ml lysis buffer (500 mM NaCl, and 50 mM EDTA) and 4% SDS (Sodium Dodecyl Sulphate) which was followed by ammonium acetate precipitation to remove the impurities and then by isopropanol for nucleic acid precipitation. Removal of RNA and protein was done by treating the genomic DNA by 2µl DNase free RNase (10mg/ml) and 15 µl proteinase K, respectively. Further purification was done by using the QIAamp spin columns. The eluted DNA was stored at -20°C. Only modification done from the original protocol was that zirconium sterile beads were replaced by the use of sterile glass beads and vortexed for 5 minutes for more convenient and efficient DNA extraction similar to Kojima *et al* (2002).

2) Protocol 2 (Kreader, 1995)–In this method 0.50 gm of faecal sample was dispersed in 25 ml of 50 mM sodium phosphate (pH 7.5) by kneading in a plastic zip-lock pouches, centrifuged and bacterial fraction was resuspended in 50 mM Tris-HCl, 50 mM Na-EDTA (pH 8.0). DNA was extracted by proteinase K lysis followed by CTAB (Cetyltrimethyl ammonium bromide) and phenol-chloroform-isoamylalcohol extractions. DNA in supernatant was precipitated by ice cold isopropanol and dissolved in TE (Tris EDTA) buffer and stored at -20°C after washing with 70%

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ethanol. However, further the DNA purification by glass method was not performed.

3) Protocol 3 (Kojima *et al,* 2002)- The DNA isolation from this method was done without any modification. The 0.50 gm faecal sample was suspended in a mixture of lysis buffer (1 ml) and organic solvent (2 ml) with addition of 9 gm glass beads. After recovering community DNA from centrifugation it was precipitated using isopropanol and dissolved in TE buffer and stored at -20°C.

4) Protocol 4 (QIAamp® DNA stool mini kit)- This method is a commercially available DNA stool kit (Qiagen, GmbH, Hilden, Germany). DNA was extracted according to manufacturer's guidelines. Briefly 0.25 gm of faecal sample was homogenised in lysis buffer having chaotropic salts, proteinase K for the enzymatic digestion and thermal lysis of the bacterial cell by incubating them at 95°C for 10 minutes. DNA bound to the spin column was stored at -20°C.

The frozen samples were thawed before the extraction of community DNA in protocol 1, 2 and 3 and were scratched for weighing in protocol 4.

The purity and concentration of DNA was determined by using Implen Nanophotometer® (Implen, GmbH, Munich, Germany). The quality of the total bacterial DNA was assessed by 0.8% agarose gel stained with ethidium bromide, visualised on a UV transilluminator and photographed (UVP BioImaging system, UVP LLC, Upland, CA).

Results

The community DNA was isolated from fresh and frozen camel faecal samples using the 4 protocols mentioned above. The quality of isolated DNA is shown in figs 1 and 2. DNA isolated from both fresh and frozen camel faecal sample using protocol 4, showed a band of high molecular weight on 0.8% agarose gel. Isolated DNA from protocol 1 showed smearing for fresh and frozen camel faecal samples and for protocol 2 smearing was seen only in fresh faecal sample. Fresh and frozen faecal samples isolated from protocol 3 did not show smearing on 0.8% agarose gel. The time taken from processing of samples to their DNA extraction was 180 minutes for protocol 1, 60 minutes for protocol 2, 30 minutes for protocol 3 and 90 minutes using protocol 4. However, these processing times did not include the preparation of the solutions. The purity and yield of the isolated DNA as indicated by measuring the concentration as well as ratio of absorbance at 260/280 nm was high (≥ 1.8) for both fresh and frozen faecal samples isolated from protocol 4 indicating that the DNA isolated is pure and in good quantity and the method was suggestive for community DNA extraction from camel faeces, whereas this was low for fresh and frozen faecal samples isolated from protocol 1, 2 and 3 (Tables 1 and 2). The similar results were obtained from both types of samples repeated with all the four protocols.

Discussion

Detection of microbes using conventional culturing techniques is laborious, lengthy and difficult for many uncultivable bacteria (Zengler *et al,* 2008). The onsite detection and isolation methods of DNA always have an added advantage in terms that the community DNA can be further amplified with specific genes, cloned and sequenced provided that it should be PCR inhibitor free and gives high yield of DNA, but this is often a tedious process as it involves the complex microbiome of hard to lyse

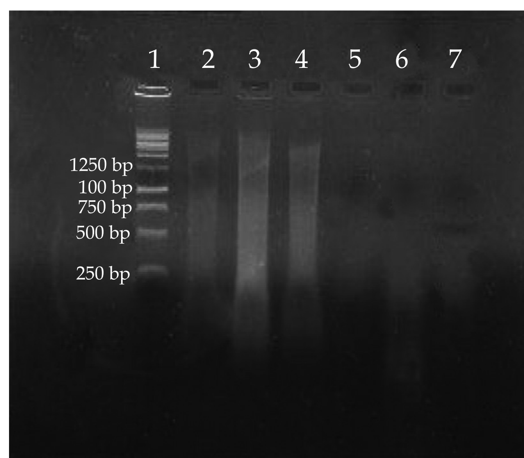

Fig 1. Lane 1- 250 bp DNA marker, Lane 2, 4, 6-DNA from protocol 1, 2, 3 for fresh faecal samples, respectively, Lane 3, 5, 7- DNA from protocol 1, 2, 3 for frozen faecal samples, respectively.

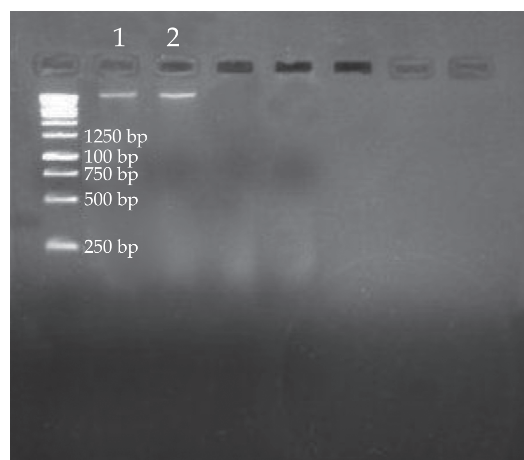

Fig 2. Lane 1- 250 bp DNA markerm, Lane 2, 3-DNA from protocol 4 for fresh and frozen samples, respectively.

bacteria, removal of phenolic compounds which may acts as PCR inhibitor. Hence the protocols are to be optimised for rapid, reliable and efficient DNA extraction from faeces of ruminants. Earlier protocols for DNA isolation are published involving the use of chemicals, enzymes, incubation at high temperatures and mechanical shearing for the lysis of the bacterial cell wall or combination of any of the methods (Jalava and Jalava, 2002). However, certain limitations lie with most of the DNA extraction method.

The first protocol tried in this study was a combination of DNA extraction using bead beating and purification using spin columns. The sterile glass beads were used instead of zirconium beads and mixed on vortexer for 5 minutes. This might have resulted in the shearing of the community DNA and resulted in its damage besides adding some minutes extra to the protocol. The protocol 2 which was a combination of chemical lysis, followed by treatment with enzyme and precipitation by isopropanol resulted in smearing of the DNA from fresh faecal sample and no observable DNA band from frozen faecal sample. This might be the result of not performing glass purification. The protocol 3 involved a single step extraction and purification without phenol, but the DNA obtained was in less concentration and no bands of DNA were observed for both fresh and frozen faecal sample. Among all the 4 protocols tried, protocol 4 showed better results both qualitatively and quantitatively and in terms of rapidity. It does not involve traditional phenol chloroform extraction which requires further clean up procedures to remove traces of phenol which may act as PCR inhibitors. The QIAamp® kit was previously found most effective extraction method to detect methanogens in community DNA (Kumar *et al,* 2011) from bovine faeces, community bacterial DNA from swines (Li *et al,* 2003; Tang *et al,* 2008; Ruiz and Rubio, 2009), mammalian faeces (McOrist *et al,* 2002). Our results are in agreement with studies reporting the commercial QIAamp Stool Kit having high extraction efficiency and PCR-compatibility among the various tested methods for human community DNA (Scupham *et al,* 2007; Nechvatal *et al,* 2008).

It can be concluded that QIAamp® DNA stool mini kit can effectively be used for isolation of

Table 1. The 260/280 ratio and DNA concentration (µg/ml) measured on nanophotometer for fresh faecal samples.

Fresh faecal Samples		Protocol 1		Protocol 2		Protocol 3		Protocol 4	
		260/280 ratio	Conc. (µg/ml)	260/280 ratio	Conc. (µg/ml)	260/280 ratio	Conc. (µg/ml)	260/280 ratio	Conc. (µg/ml)
1	A	1.74	48	1.76	60	1.20	40	1.84	80
	*	1.70	40	1.68	44	1.26	42	1.87	82
2	A	0.98	34	0.80	32	1.08	36	1.91	90
	*	1.0	36	1.02	34	1.11	36	1.89	84
3	A	1.10	37	1.32	40	1.0	34	1.88	85
	*	1.06	36	1.36	42	0.76	29	1.89	84
4	A	1.65	52	1.75	58	0.99	33	1.92	94
	*	1.59	50	1.73	49	0.95	30	1.94	95

* - same samples repeated for all the 4 protocols.

Table 2. The 260/280 ratio and DNA concentration (µg/ml) measured on nanophotometer for frozen faecal samples.

Frozen faecal Samples		Protocol 1		Protocol 2		Protocol 3		Protocol 4	
		260/280 ratio	Conc. (µg/ml)	260/280 ratio	Conc. (µg/ml)	260/280 ratio	Conc. (µg/ml)	260/280 ratio	Conc. (µg/ml)
1	A	1.57	52	1.42	43	1.39	40	1.82	80
	*	1.49	50	1.40	40	1.30	37	1.86	82
2	A	1.27	43	1.38	39	1.28	37	1.90	90
	*	1.38	37	1.25	40	1.22	41	1.88	86
3	A	0.99	39	1.72	49	0.76	30	1.91	89
	*	1.0	38	1.69	46	0.80	32	1.87	82
4	A	1.72	50	1.52	42	1.24	40	1.98	95
	*	1.68	48	1.49	30	1.28	42	1.96	94

* - same samples repeated for all the 4 protocols.

community DNA which invariably gave the best results both from fresh and frozen faeces of camel.

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