JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2013, Vol. 1, No. 5, pp:392-395
Available at www.jcrs010.com

ORIGINAL ARTICLE

SOMATIC CELL COUNTS POSITIVE EFFECTS ON THE DNA YIELD EXTRACTED DIRECTLY FROM MURRAH BUFFALO MILK

Feng Li Yang, Xiao Shan Li
College of Animal Science, Yangtze University, Jingzhou, 434025 P.R. China

ABSTRACT: The aim of this study was to verify the relationship between somatic cell counts (SCC) and the DNA yield extracted directly from buffalo milk. Twenty-five Murrah buffalo milk samples were measured SCC and extracted DNA. Milk SCC was between 16,000 and 1,934,000 cells/mL. DNA yield was within the range from 3747.2 ng/µl to 7080.7 ng/µl. The ratio of A260/A280 and A260/A230 of genomic DNA was ranged from 1.66 to 2.17 and 1.71 to 2.13, respectively. The results indicated that DNA can be extracted directly from Murrah Buffalo milk, but may be presence of some contaminants. The Pearson's correlation analysis showed a very strong positive correlation between the DNA yield extracted directly from buffalo milk and the milk SCC ($r = 0.927$, $P < 0.001$). Milk sample was preferred to blood as a source of DNA, due to milk collection was routinely performed, as well as less expensive and more easily accomplished than blood collection. Moreover, milk collection was also less stressful to animals, given that capture, handling, and venipuncture were not required for milk sampling.
KEYWORDS: Buffalo milk, Somatic cell count, Genomic DNA.

INTRODUCTION

Milk somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (leukocytes) that have entered the mammary gland in response to injury or infection (Sharma *et al.,* 2011). The milk somatic cells include 75% leukocytes (i.e. neutrophils, macrophages, lymphocytes, erythrocytes, which are derived from blood circulation) and 25% epithelial cells. While, the proportions of neutrophils), macrophages, and lymphocytes for healthy milk are approximately 12, 60, and 28%, respectively (Kelly *et al.,* 2000). The epithelial cells of the glands are normally shed and get renewed, however, during infection the numbers increase. The normal composition of milk somatic cells varies with the type of secretion or lactation cycle. Normally, in cows' milk from a healthy mammary gland, the SCC is lower than 100,000 cells/mL, while bacterial infection can make it increase to above 1,000,000 cells/ml (Bytyqi *et al.,* 2010), the stage of lactation, season, milk yield and number of lactations are all also known to influence milk SCC (Kelly *et al.,* 2000). Whole blood can contain up to 350 times more nucleated cells than milk, when comparing lower cell counts for the same volumes of whole blood and milk (Murphy *et al.,* 2002). Genetic research has recently shown a marked increase as interest in understanding the genetic basis of diseases and drug regimens increases. Almost of all studies require DNA isolations. Blood leukocytes are generally used as an excellent source of genomic DNA. Nevertheless, collection of blood samples causes technical difficulties and leads to stress for animals. Other tissues, such as skin biopsies or hair follicles, can be also used as a source of genomic DNA (Healy *et al.,* 2002). However, milk, which is widely available and obtained noninvasively, is the source of choice in lactating animals, and does not require trained personnel or the milker. Several studies have developed methods to extract genomic DNA from somatic cells of bovine milk (Amills *et al.,* 1997; Lipkin *et al.,* 1993; Murphy *et al.,* 2002), caprine milk (D'Angelo *et al.,* 2007; Lopez-Calleja *et al.,* 2004), and human milk (Abdalla *et al.,* 2009; Haas *et al.,* 2011). However, there is scarcity of literature on the relationship between milk SCC and DNA extracted directly from milk samples. Therefore, the purpose of the present study was to evaluate the effects of SCC on the DNA yield extracted directly from Murrah buffalo milk.

MATERIAL AND METHODS

2.1. Samples

Twenty-five Murrah Buffaloes at 5 to 8 weeks postpartum, in their second to fourth lactation feeding in Guangxi Buffalo Research Institute. All the buffaloes did not show clinical signs of mastitis or other illnesses. After a quarter had been washed with tap water and dried, the teat

Corresponding Author: Feng Li Yang, College of Animal Science, Yangtze University, Jingzhou, P.R. China.
E-mail: fengli168@qq.com

end was disinfected by wiping with cotton wool soaked in 70% ethyl alcohol. Milk samples were collected prior to the morning milking into sterile test tubes (10 ml) hand-stripping after discarding the first three squirts of milk, and then placed in an icebox and transported to the laboratory for examination within one hour after collection. Milk sampling was done only once from each animal.

2.2. Somatic Cell Count

Milk SCC was measured quantitatively using the Fossomatic Minor analyzer (Foss Electric, Hillerød, Denmark) according to the International Dairy Federation standard (IDF, 1995).

2.3. DNA Extraction

The total DNA was extracted from milk samples according to the previously described method with some modifications (De *et al.*, 2011; Lopez-Calleja *et al.*, 2004). Briefly, 0.5 mL of normal saline solution was added to 1 mL of the milk sample. Samples were mixed by inverting them 10 times before they were centrifuged at 13,000 rpm for 10 min at 4°C. The result was a cream pad on the top of a clear supernatant. Both the pad and the supernatant were carefully removed, and the pellet left at the bottom of the tube was resuspended in 1 mL of normal saline solution, then the mixture was centrifuged again and the pad on the top and the supernatant were carefully discarded. The resulting pellet, which contained cells and caseins, was resolved in 860 µL of TES buffer (pH 8.0; 50 mM Tris-HCl, 10 mM EDTA, and 1% SDS), 100 µL of 5 M guanidine hydrochloride, and 40 µL of 20 mg/mL proteinase K. The mixtures were incubated in a water bath at 50°C overnight, and they were left to cool at room temperature. The cellular debris and proteins were removed by adding 500 µL Tris saturated phenol, and the inverted those 10 times before centrifugation at 13,000 rpm for 5 min at 4°C. The clear aqueous supernatant obtained after the centrifugation was carefully transferred to a new Eppendorf tube. The DNA was precipitated by adding 0.8 volume of isopropanol in the presence of 0.1 volume of 3 M sodium acetate (pH 5.3). The DNA pellet, obtained after centrifugation for 10 min at 13,000 rpm, was air dried and subsequently dissolved in 100 µl of sterile double distilled water.

2.4. Spectrophotometer measurements

Quality of the DNA extracted was determined by a UV-VIS spectrophotometer (Nanodrop, Thermo Scientific, USA). The concentration of DNA was calculated based on the approximation that an absorbance reading of 1 of the purified DNA at 260 nm was taken to correspond to 50 ng/µl. The ratio of absorbance at 260 nm and 280 nm was used to assess protein or RNA contamination while the ratio of absorbance at 260 nm and 230 nm was calculated to assess organic solvent contamination (Turashvili *et al.*, 2012). Both spectrophotometer measurements constituted criteria for DNA quality assessment with higher values associated with better DNA quantity and purity. An A260/A 280 ratio of 1.8-2.0 is indicative of high purity (Pirondini *et al.*, 2010).

2.5. Statistical analysis

Statistical analyses were carried out using SPSS software, version 19.0 for windows (SPSS Inc., Chicago, IL, USA). Pearson's correlation coefficients and linear regression were used to investigate the relationship between milk SCC and DNA concentration extracted. Differences were considered significant when $P<0.01$.

RESULTS AND DISCUSSION

The SCC, concentrations and purities of the DNA extracted from the 25 Buffalo milk samples, as determined using absorbance values at 230 nm (A230), 260 nm (A260), and 280 nm (A280), are presented in Table 1. Milk SCC of the samples was between 16,000 and 1,934,000 cells/mL. DNA yield obtained was within the range of 3747.2 ng/µl to 7080.7 ng/µl. The ratio of A260/A280 and A260/A230 of genomic DNA was ranged from 1.66 to 2.17 and 1.71 to 2.13, respectively.

Table 1: The milk SCCs, DNA yield and UV parameters

No.	SCC (10^3)	DNA yield (ng/µl)	A260	A280	A260/A280	A260/A230
1	56	4052.4	81.05	44.03	1.84	1.98
2	160	4492.6	117.85	62.61	1.88	1.84
3	1859	6753.1	135.06	79.21	1.71	1.95
4	343	4613.8	88.28	46.54	1.9	2.03
5	16	4394.7	95.9	52.23	1.84	1.78
6	71	4279.1	57.58	26.48	2.17	2.13
7	38	4293.5	95.87	51.15	1.87	1.85
8	66	4428.9	96.58	50.86	1.9	1.92
9	219	4430.1	84.6	46.67	1.81	1.82
10	49	4245.4	84.91	45.88	1.85	1.87

11	24	4603.1	92.06	49.26	1.87	1.82
12	251	4720.5	102.41	53.37	1.92	1.86
13	55	4869.3	109.39	57.06	1.92	1.94
14	30	3747.2	69.94	39.6	1.77	1.71
15	44	4100.5	82.01	43.91	1.87	1.87
16	128	3877.8	67.56	37.83	1.79	1.94
17	123	4680.8	95.62	50.36	1.9	1.88
18	91	4299.2	73.98	40.58	1.82	1.94
19	273	4863.8	97.28	51.88	1.88	1.84
20	153	4553.5	93.07	50	1.86	1.91
21	121	4239.4	84.79	44.8	1.89	2.08
22	182	4987.5	109.75	57.02	1.92	1.91
23	133	3916.1	78.32	41.97	1.87	2.07
24	242	4220.9	60.42	29.76	2.03	2.1
25	1934	7080.7	145.61	87.68	1.66	1.72

The normal composition of milk somatic cells varies with the type of secretion or lactation cycle. The milk somatic cells include 75% leucocytes, i.e. neutrophils, macrophages, lymphocytes, erythrocytes, and 25% epithelial cells (Sharma *et al.*, 2011). Milk sample is preferred to blood as a source of DNA due to milk collection is routinely performed and is less expensive and more easily accomplished than blood collection. Moreover, milk collection is also less stressful to animals, given that capture, handling, and venipuncture are not required for milk sampling. In recent years, several studies have developed methods to extract DNA directly from milk somatic cells instead of blood and use organic extraction, overnight incubation, or expensive commercial kits (Amills *et al.*, 1997; Lipkin *et al.*, 1993; Lopez-Calleja *et al.*, 2004). A rapid simple salting-out method for DNA extraction from caprine milk was proposed but only 75% of tested samples were suitable as a substrate for PCR-RFLP genotyping (D'Angelo *et al.*, 2007). In another study, a solid phase absorption commercial kit (Wizard DNA cleanup kit, Promega) was tested in ruminant's milk with reliable results (Lopez-Calleja *et al.*, 2004). In most literatures, the methods extracted milk DNA were used low milk volumes and required overnight incubation of samples with proteinase K.

D'Angelo *et al.*, (2007) extracted the DNA from goat milk sample by a simple salting-out method, the total DNA yield ranged from 2.12 to 610.12 μg per goat milk sample (40 mL of raw milk), and SCC for these samples ranged from 79,000 to 4,000,000 cells/mL. While, in this study, the method extracted DNA from milk samples was according to De *et al.*, (2011) and Lopez-Calleja *et al.*, (2004) with some modifications, the volume of each sample extracted for DNA is 1 mL Murrah buffalo milk, the final DNA concentrations range from 374.72 to 708.07 μg per sample, and SCC between 16,000 and 1,934,000 cells/mL.

The ratio of A260 and A280 is used to assess the purity of DNA. If the ratio is appreciably lower than 1.8, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. DNA extractions with a 260/280 ratio of above 1.80 are deemed to be of high quality (Anonymous, 2008). A260/A280 ratio values ranged from 1.66 to 2.17 in this study, with 84% of samples tested achieving a value of 1.8 or greater (Table 1). The ratio of absorbance at 260 nm and 230 nm is used as a secondary measure of nucleic acid purity. The A260/A230 values for "pure" nucleic acid are often higher than the respective A260/A280 values (Anonymous, 2008). Values of 2 or more would be considered high quality samples, less than this indicates the presence of contaminants that absorb light at 230 nm, such as carbohydrates, guanidine thiocyanate, phenols and humic acids (Eland *et al.*, 2012). In the samples, 260/230 ratios values ranged from 1.71 to 2.13, with 20% of samples tested achieving a value of 2 or greater (Table 1). Therefore, the DNA extracted directly from Murrah Buffalo milk samples may be presence of contaminants, which need confirmed in the future.

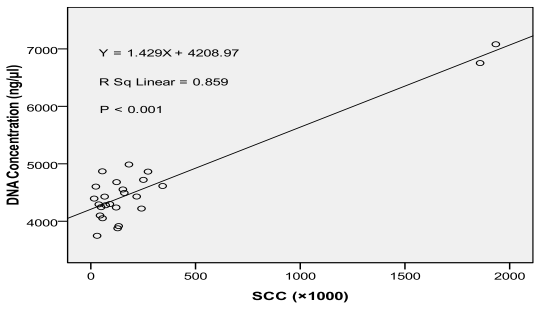

Figure 1: Correlation analysis between milk SCC and DNA concentration extracted directly from buffalo milk.

The Pearson's correlation analysis showed a very strong positive correlation between the DNA yield extracted directly from buffalo milk and the milk SCC (r= 0.927, P<0.001). The linear

regression equation was y= 1.429 x + 4208.97, where x and y represent milk SCC and DNA yield, respectively (Figure 1). The significant positive correlation (P<0.001) between milk SCC and DNA extracted from buffalo milk was demonstrated in the present study, which is consistent with the research by D'Angelo *et al.* (2007) who reported that a positive correlation (P<0.001) was found between DNA yield and SCC in goats' milk. The result indicated that most of the DNA extracted from milk originated from SCC. However, further studies are required to confirm the composition of DNA extracted.

CONCLUSION

The result of the study indicated that DNA can be extracted directly from Murrah Buffalo milk, and a very strong positive correlation between the DNA yield extracted directly from buffalo milk and the milk SCC (r= 0.927, P<0.001). Milk sample was preferred to blood as a source of DNA, due to milk collection was routinely performed, as well as less expensive and more easily accomplished than blood collection.

ACKNOWLEDGEMENTS

The study was supported by Guangxi Department of Science and Technology (No. 2012GXNSFBA053071).

REFERENCES

Abdalla SF, Musa OA, Aradaib IE. Evaluation of Milk as a Source of Human DNA in Lactating Women Using Polymerase Chain Reaction. International Journal of Molecular Medicine and Advance Sciences 2009;5:6-9.

Amills M, Francino O, Jansa M, Sanchez A. Isolation of genomic DNA from milk samples by using Chelex resin. Journal of Dairy Research 1997;64:231-238.

Anonymous. 2008. 260/280 and 260/230 ratios. T009 technical bulletin. Thermo Scientific. http://www.phenogenomics.ca/transgenics/docs/NanoDrop%20Nucleic-Acid-Purity-Ratios.pdf (2011 Aug. 29).

Bytyqi H, Zaugg U, Sherifi K, Hamidi A, Gjonbalaj M, Muji S, Mehmeti H. Influence of management and physiological factors on somatic cell count in raw milk in Kosova. Veterinarski Archiv 2010;80:173-183.

D'Angelo F, Santillo A, Sevi A, Albenzio M. Technical note: A simple salting-out method for DNA extraction from milk somatic cells: investigation into the goat CSN1S1 gene. Journal of Dairy Science 2007;90:3550-3552.

De S, Brahma B, Polley S, Mukherjee A, Banerjee D, Gohaina M, Singh K P, Singh R, Datta R K, Goswami S L. Simplex and duplex PCR assays for species specific identification of cattle and buffalo milk and cheese. Food Control 2011;22:690-696.

Eland LE, Davenport R, Mota CR. Evaluation of DNA extraction methods for freshwater eukaryotic microalgae. Water Research 2012;46:5355-5364.

Haas DM, Daum M, Skaar T, Philips S, Miracle D, Renbarger JL. Human breast milk as a source of DNA for amplification. The Journal of Clinical Pharmacology 2011;51:616-619.

Healy PJ, Dennis JA, Windsor PA, Pierce KD, Schofield PA. Genotyping cattle for inherited congenital myoclonus and maple syrup urine disease. Australian Veterinary Journal 2002;80:695-697.

IDF. Milk Enumeration of Somatic Cells. Standard no. 148A. International Dairy Federation, Brussels, Belgium. 1995.

Kelly AL, Tiernan D, O'Sullivan C, Joyce P. Correlation between bovine milk somatic cell count and polymorphonuclear leukocyte level for samples of bulk milk and milk from individual cows. Journal of Dairy Science 2000;83:300-304.

Lipkin E, Shalom A, Khatib H, Soller M, Friedmann A. Milk as a source of deoxyribonucleic acid and as a substrate for the polymerase chain reaction. Journal of Dairy Science 1993;76:2025-2032.

Lopez-Calleja I, Gonzalez I, Fajardo V, Rodriguez MA, Hernandez P E, Garcia T, Martin R. Rapid detection of cows' milk in sheeps' and goats' milk by a species-specific polymerase chain reaction technique. Journal of Dairy Science 2004;87:2839-2845.

Murphy MA, Shariflou MR, Moran C. High quality genomic DNA extraction from large milk samples. Journal of Dairy Research 2002;69:645-649.

Pirondini A, Bonas U, Maestri E, Visioli G, Marmiroli M, Marmiroli N. Yield and amplificability of different DNA extraction procedures for traceability in the dairy food chain. Food Control 2010;21:663-668.

Sharma N, Singh NK, Bhadwal MS. Relationship of somatic cell count and mastitis: An overview. Asian - Australasian Journal of Animal Sciences 2011;24:429-438.

Turashvili G, Yang W, McKinney S, Kalloger S, Gale N, Ng Y, Chow K, Bell L, Lorette J, Carrier M, Luk M, Aparicio S, Huntsman D, Yip S. Nucleic acid quantity and quality from paraffin blocks: defining optimal fixation, processing and DNA/RNA extraction techniques. Experimental and Molecular Pathology 2012;92:33-43.