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Research Article

BANDING PATTERN INDUCED BY RESTRICTION ENDONUCLEASES ON BUFFALO (BUBALUS BUBALIS) CHROMOSOMES

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ABSTRACT

The karyotype of reverine buffalo is composed of 24 pairs of autosomes and a pair of sex chromosomes (2n=50) The first five pairs of autosomes are submetacentric where as all other chromosomes are acrocentric including sex chromosomes, which are many times difficult to distinguish individually, even in well-banded chromosomes. The metaphase chromosomes preparations are susceptible to restriction enzymes. The present study was conducted to generate restriction enzyme banding profile on buffalo chromosomes to identify the homologous chromosome and structural abnormalities in addition to the conventional G-, R-, and C- banding. Chromosomal slides prepared by whole blood culture techniques were treated with various restriction enzymes; *MboI, HinfI, HaeIII, AvaI, AvaII* and *TaqI,* which revealed G- and C-like bands on buffalo chromosomes. Restriction enzymes treated cause the loss of DNA which is directly related to a decreased Giemsa stain that generates a characteristic staining pattern through out the length of the chromosomes. The protocols, quantity of enzyme used, banding pattern produced on chromosomes and preparation of karyotypes have been discussed in the present article.

Key words: Chromosomes, karyotype, restriction enzyme, DNA, banding

Chromosomal aberrations associated with reduced fertility have caused concern in various countries and many of them have been screening their breeding animals (Kovacs and Szepeshelyi, 1987, Patel, 2000). It is known that several domestic animals have chromosomes, which are very difficult to distinguish individually. In buffaloes most of autosomes and sex chromosomes are acrocentric in structure, therefore, identification problems are encountered while comparing with international standard (Gustavsson, 1999). As a result, many aberrations particularly structural that do not change the shape and size of chromosomes, have escaped our attention. A few autosomal reciprocal translocations (Kovacs *et al.*, 1992) and inversions (Roldan *et al.*, 1984) have so far been found in cattle, probably due to identification problems. Identification of reciprocal translocations not producing drastic changes in length/shape is difficult even in well-banded chromosomes preparations. The metaphase chromosome preparations are susceptible to restriction enzymes (Alfi *et al.*, 1973). The present study was conducted to generate restriction enzyme banding profile through out the length of chromosomes in buffalo using the enzymes: *Mbo* I, *Hinf* I, *Hae* III, *Ava* I, *Ava II* and *Taq* I so that these bands could help to identify the homologous chromosomes and structural abnormalities in addition to conventional G-, R- and C– bandings.

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MATERIALS AND METHODS

Lymphocyte culture: Chromosome preparation was made by using standard whole blood culture in RPMI-1640 medium supplemented with antibiotics, 15% fetal calf serum and pokeweed mitogen as described by Patel (1999).

Restriction endonuclease/ Giemsa banding: As metaphase chromosomes prepared by methanol-acetic acid fixation and air-drying technique are susceptible to DNases (Alfi *et al.*, 1973), 40 µl to 60 µl solution containing 0.8 unit/ il of restriction enzyme and 10X assay buffer provided with enzyme, was placed on a slide,

overlaid with a cover glass (24 x 60 mm), and incubated at 37°C in a pre-warmed humid chamber. Incubation time ranges from 18 to 24 hours depending on the enzyme batch and the age of the slide. At the end of the incubation period, the slides were rinsed thoroughly in deionized water and allowed to dry. The slides were stained with 2% Giemsa at room temperature for 10 to 12 minutes and examined under light microscope. The *Mbo* I, *Hinf* I, *Hae* III, *Taq* I, *Ava* I and *Ava* II restriction enzymes (RE) were used to generate possible bands in buffalo chromosomes by treating the slide with these enzymes at various time intervals as mentioned in Table 1.

Table 1

Restriction enzymes used for generating bands on buffalo chromosomes

Restriction enzyme*	Enzyme conc. (units)	Enzyme conc. (unit/µl)	Incubation temp(°C)	Incubation time
Mbo I	32/40 µl	0.8	37	18
Mbo I	32/40 µl	0.8	37	24
Hinf I	32/40 µl	0.8	37	18
Hinf I	32/40 µl	0.8	37	24
Hae III	48/60 µl	0.8	37	18
Hae III	48/60 µl	0.8	37	20
Hae III	48/60 µl	0.8	37	24
Taq I	32/40 µl	0.8	65	08
Taq I	32/40 µl	0.8	65	18
Ava I	32/40 µl	0.8	37	24
Ava II	30/50 µl	0.6	37	24

* All enzymes were from Bangalore Genei Pvt. Ltd. India and *Ava II* from Promega, USA.

Karyotyping: For preparing the karyotype of the chromosomes treated with each enzyme, photographs of best field were taken. The chromosomes then were cut and the approximate length was measured by Vernier calipers. The twisted chromosomes were first measured with a thread and then in turn with the Vernier calipers. The chromosomes were finally arranged considering their length in descending order and by their banding pattern.

RESULTS AND DISCUSSION

The reverine buffalo (*Bubalus bubalis*) possesses 50 (2n) chromosomes. The karyotype is composed of 24 pairs of autosomes and sex chromosomes as XY in male and XX in female. The first five pairs of autosomes are submetacentric where as all other chromosomes are acrocentric including sex chromosomes. The X – chromosome is the largest acrocentric chromosome that could be easily identified. The Y- chromosome is among the smaller acrocentric chromosome (Kumar and Yadav, 1991) that can be identified by conventional G- banding.

Restriction endonucleases (REs) are known to induce DNA cleavage in the fixed metaphase chromosomes (Sahasrabuddhe *et al.*, 1978) that can be revealed as characteristic staining patterns. Restriction enzymes can produce large/ small fragment of DNA depends on recognition sequence when deposited on chromosomes. The loss of DNA is directly related to a decreased Giemsa staining that generates a characteristic staining pattern in metaphase chromosomes (Bianchi *et al.*, 1985, Miller *et al.*, 1983). It is further suggested that DNA fragments longer than one kilo base pair (Kb) remain in the chromatin, where as smaller fragments of about 200 base pairs get extracted from the chromosomes (Miller *et al.*, 1983, 1984). The enzyme, which induces C- like bands, removes a significant amount of DNA from the chromosomes, except from certain C- band regions, where as *Hae* III, which produces G- and C- like bands, removes a lesser amount of DNA.

The slides with metaphase spread was treated with various RE, stained with Giemsa, screened under oil immersion, photographed and karyotyped based on the length of chromosomes. The description of the reaction and banding pattern induced by each enzyme on buffalo chromosomes are given below

***Mbo* I**: Chromosomes treated with 32 units of *Mbo* I enzyme for 24 h at 37°C, revealed small dark bands at the primary constriction region of some of the acrocentric chromosomes and most of all the chromosomes including X-chromosome showed darkly stained bands through out the length of chromosomes similar to G- bands as shown in karyotype (Figs 1, 2). However, Y- chromosome showed a small band on primary constriction as well as terminal ends. It seems that the number of dark bands are less than G- bands produced by trypsin. In human, metaphase chromosomes treated with *Mbo* I shows an overall reduction in staining except for some of the C- band regions. Chromosome

numbers 3, 5, 7, 10, 12, 18 and 20 show darkly stained centromeric bands (Verma and Babu, 1989).

***Hinf* I**: Chromosome slides treated with 32 units of *Hinf* I enzyme for 24 h at 37°C revealed a typical dark band or paracentromeric dark band on each chromosome except sex chromosomes where these bands were visible near primary constriction. The possible karyotype was prepared as per the length of chromosomes. It was easy to identify 1st five pair of chromosomes because of their submetacentric shape and X-chromosome because of the longest length. However, other chromosomes were paired as per length and position of typical dark band. Some of the human chromosomes also show paracentromeric dark bands, which apparently reflect the heteromorphic segments of corresponding C-bands. Similarly, the satellite regions and proximal short arm regions of some of the acrocentric chromosomes show prominent bands. (Verma and Babu, 1989). In bovine *Hinf* I treatment and Giemsa stained metaphase showed similar heterochromatin blocks on chromosomes (Hidas, 1995).

***Hae* III:** Chromosome slides treated with 48 units of *Hae* III enzyme for 24 h at 37°C, induced differential staining along the length of the chromosomes similar to G-bands, and bands at the centromeric regions of chromosomes. These were not as prominent as compared to C- bands induced by barium hydroxide method. A G-like band pattern was observed in chromosomes of cattle and buffalo by Tomar and Goswami (1998). They also observed C-like bands simultaneously with G-like bands on some of chromosomes of cattle, but none of buffalo chromosomes could reveal C-like bands with this enzyme. It could be due to insufficient period of treatment of chromosome slides (6-18 hrs) with *Hae* III enzyme. Similar to cattle and buffalo, human chromosomes also revealed differential staining along the length of the chromosomes similar to the G-bands and prominent dark bands at the centromeric regions of a few chromosomes (Verma and Babu, 1989).

***Ava* I:** Slides of metaphase chromosomes treated with 32 units of *Ava* I enzyme for 24 h at 37°C, induced differential staining along the length of the chromosomes. Similar to G-bands as terminal bands were observed on q arms of chromosomes number 3, 6, 7, 10, 16, 18, 23, X and Y. Some of the acrocentric chromosomes have shown partial to prominent C- like bands. Because of the paucity of literature on other animal species as well as humans, it was not possible to compare the *Ava* I induced bands of buffalo with these species.

***Ava* II:** Chromosomes treated with 30 units of *Ava* II enzyme for 24 h at 37°C, revealed dark and light bands through out the length of chromosomes similar to G- bands (Fig 3).) The karyotype was prepared as per the length of chromosomes and bands on homologous chromosomes (Fig 4)). Like *Ava* I treatment, *Ava* II enzyme could not reveal the centromeric bands in any of chromosomes. Due to the paucity of literature on other species of animals and human beings, it was not possible to compare the *Ava* II induced bands of buffalo with these species.

***Taq* I:** Chromosome slides treated with 32 units of *Taq* I enzyme for 8 h at 65°C revealed moderate C - like bands in almost all chromosomes (Fig 5). For karyotyping, the chromosomes were paired in order of decreasing length, except sex chromosomes (Fig 6). However, *Taq* I induced moderate overall digestion of chromosomes of cattle without any characteristic pattern (Hidas, 1995). Subsequent alkaline C- banding induced differential staining of some heterochromatin regions, which is characteristic for the conventional C-banding. Nevertheless, the pattern obtained by restriction enzymes and C- banding showed considerable difference from the conventional C- banding. The difference between banding patterns generated by *Taq* I in present study and observations of Hidas (1995), may be due to the duration of the enzyme treatment. Because of the paucity of literature on effect of *Taq* I on human chromosomes, it was not possible to compare the banding pattern with human chromosome.

Most of enzymes produced banding pattern through out the length of chromosomes which were similar to conventional g- or C-banding. Restriction enzyme produced bands can be applied where identification of chromosomal abnormalities are doubtful. The techniques can be further improved by modifications in above protocols.

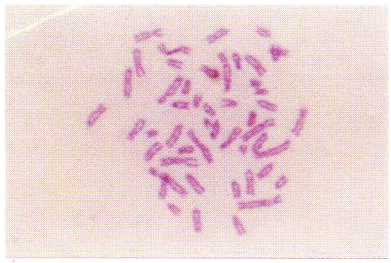

Fig 1. *Mbo* I-treated metaphase chromosomes of male buffalo.

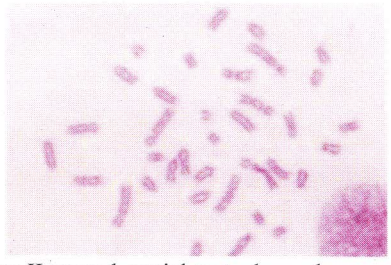

Fig 3. Ava II-treated partial metaphase chromosomes of male buffalo.

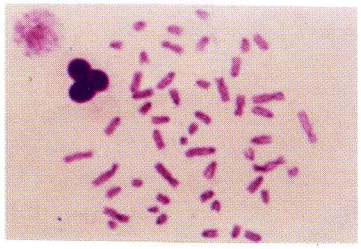

Fig 5. *Taq* I-treated metaphase chromosomes of male bufalo

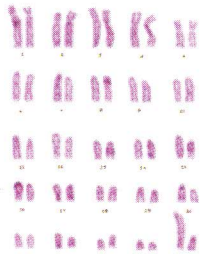

Fig 2. Karyotype of *Mbo* I-treated metaphase chromosomes of male buffalo.

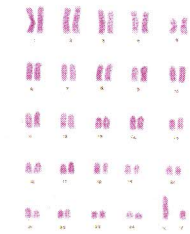

Fig 4. Karyotype of Ava II-treated metaphase chromosomes of male buffalo.

Fig 6. Karyotype of *Taq* I-treated metaphase chromosomes of male buffalo.

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