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COMPARATIVE GENE FREQUENCIES OF NUCLEOSIDE PHOSPHORYLASE FROM CATTLE OF *BOS TAURUS* AND *BOS INDICUS* DERIVATION IN BRAZIL

LUCIA PANEPUCCI,* MAURICIO M. DE ALENCAR,* VERA VICENTE* and NORMA MORTARI†
*EMBRAPA-UEPAE de São Carlos, Cx. Postal 339, 13560 SP, Brazil (Tel. (0162) 71-6123); and
†Universidade Federal de São Carlos, SP, Brazil

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Abstract—1. Two nucleoside phosphorylase (NP) phenotypes were detected in 844 animals from four distinct genetic groups of *Bos taurus* and *Bos indicus* derivation.

2. *Bos indicus* breeds like Guzerat (Kankrej), Gir, Nellore (Ongole) and Indubrazil presented an NP-H frequency of 1.00, 0.928, 0.776 and 0.754 respectively, while the Canchim breed, a *Bos taurus–Bos indicus* crossbred cattle (5/8 Charolais–3/8 Zebu) presented a frequency of 0.372.

3. The high frequency detected from the NP-H allele in the *Bos indicus* breeds strongly suggests that this enzyme is a genetic marker for cattle and that it probably has a very high frequency in all Indian breeds.

INTRODUCTION

According to Ansay and Hanset (1972), cattle erythrocytes show two electrophoretic phenotypes of NP: NP-H (or NP-High) a large blot, and NP-L (or NP-Low) a thin line on electrophoretic patterns. They also performed an analysis of matings, indicating that the NP-L phenotype is controlled by a recessive allele. Ansay (1975) reported a new phenotype in the progeny of an NP-H bull whose characteristic blot showed a slightly higher anodal electrophoretic mobility. Briouga *et al.* (1981), Mahin and Mammad (1982), Queval and Bambara (1984) and Gonzálex *et al.* (1987) described two phenotypes, NP-H and NP-L.

Cattle of *Bos taurus* derivation show very low values of NP-H frequency (Ansay, 1975; Ansay and Hanset, 1972; Mahin and Mammad, 1982; Queval and Bambara, 1984; Gonzalez *et al.*, 1987). Queval and Bambara (1984) first noticed a slightly higher frequency of NP-H in Zebu cattle than in Baoules cattle of taurus derivation (0.4595 vs 0.3898).

Many authors have noticed an increase in the frequency of the NP-H allele when going from northern Europe to the tropics (Briouga *et al.*, 1984; Queval and Bambara, 1984). Panepucci (1989) studied 317 crossbred cattle divided into four crosses (3/8, 1/2, 5/8 and 3/4 European–Zebu) and noticed a decreasing value of the NP-H gene frequency as European blood increased.

In an attempt to elucidate the variations of NP-H frequencies encountered in the different breeds analysed, nucleoside phosphorylase distribution was studied in five genetic groups, one crossbred European–Zebu and four Zebu breeds.

MATERIALS AND METHODS

Animals

Blood samples were collected from 844 beef and dairy animals divided into five genetic groups located in the states of São Paulo and Minas Gerais, Brazil. The genetic groups were: 475 Canchim animals (5/8 Charolais–3/8 Zebu crossbred cattle); 22 Guzerat (Kankrej); 194 Gir; 120 Nellore (Ongole); and 33 Indubrazil, all of *Bos indicus* derivation. The sampling of each breed was carried out at random.

Methods

Blood samples were taken from the jugular vein, either with heparine or EDTA as anticoagulants and kept refrigerated until arrival at the Laboratory. Erythrocytic lysates were prepared and deep frozen at −20°C. Nucleoside phosphorylase was identified by starch gel (Sigma 9%) electrophoresis, after Edwards *et al.* (1971). For routine screening, electrophoresis was carried out in gels containing 13% (w/v) corn starch, prepared according to Val *et al.* (1981) or potato starch (10% w/v), in 0.05 M sodium phosphate buffer at pH 6.9 for the gel and 0.1 M, pH 6.9 for the reservoirs. In the latter system, two rows of 18 samples each were inserted in the gel, which permitted the analysis of 36 animals in one electrophoresis, and also the utilization of 15 ml of staining solution. The frequency of the NP-H allele was estimated on the assumption of Hardy–Weinberg equilibrium.

RESULTS

The starch gel electrophoresis of hemolysates disclosed two distinct patterns: NP-H, a large blot, and NP-L, a thin band (Fig. 1). A few samples of the NP-H phenotype showed faster anodal mobility suggesting the existence of two variants as described by Ansay (1975), NP-H^L and NP-H^F. No discrimination was made between these two phenotypes. The routine screening in phosphate buffer is shown in Fig. 2. Samples collected 3 years before typing proved as suitable for electrophoresis as did fresh samples. The only modification observed was that the NP-L band faded with ageing.

Five cattle breeds were surveyed for NP polymorphism. The frequencies of NP-H phenotypes and of the NP-H allele for these three populations are summarized in Table 1. The frequency of the H allele

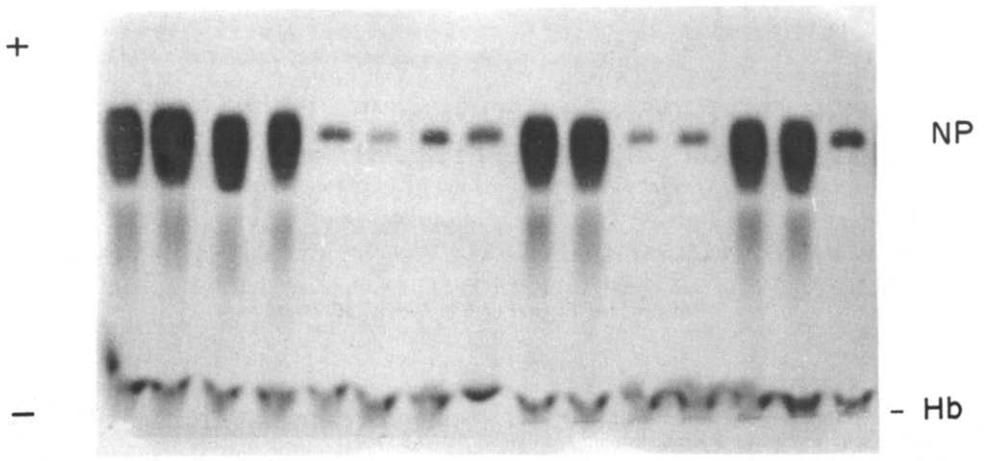

Fig. 1. Potato starch gel electrophoresis of Canchim cattle showing the two NP phenotypes detected.

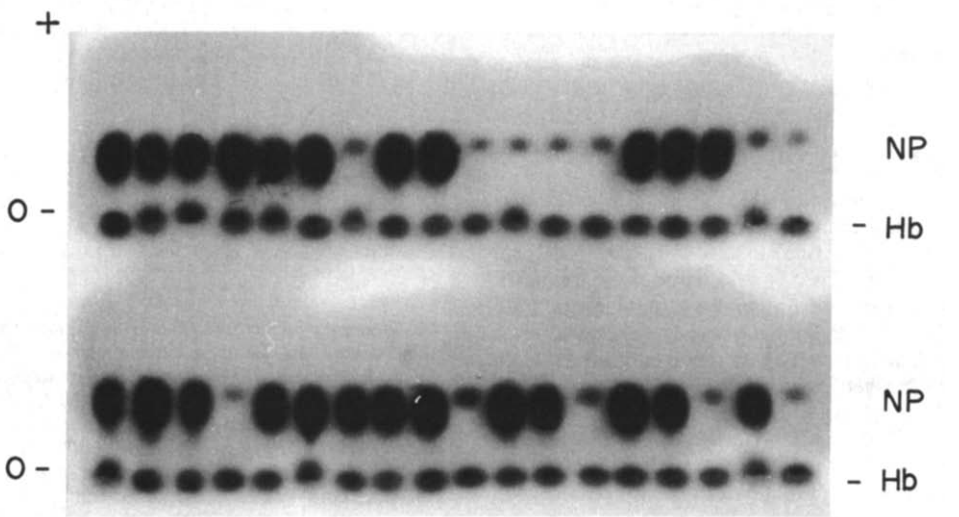

Fig. 2. Corn starch gel electrophoresis showing the routine screening in phosphate buffer, pH 6.9.

was estimated on the assumption of Hardy–Weinberg equilibrium. In the Gir cattle breed, one animal was found with the NP-L phenotype in samples from 194 animals analysed. This NP-L band had the same electrophoretic mobility and the same staining intensity as the NP-L phenotypes of the *Bos indicus* and Canchim cattle breeds. The Guzerat and Gir cattle breeds had the highest frequency for the NP-H allele followed by the Nellore (Ongole), Indubrazil and Canchim cattle breeds.

Corroborating the work of Ansay and Hanset (1972), which suggests that nucleoside phosphorylase is not sex linked, Table 2 shows the number of phenotypes for males and females and shows that the differences detected are not significant ($P > 0.05$).

Table 1. Estimated frequency of NP-H in the different breeds analysed

Breed	Number of animals	Number of NP-H phenotypes	NP-H frequency*
Canchim	475	288	0.372
Guzerat	22	22	1.00
Gir	194	193	0.928
Nellore	120	113	0.776
Indubrazil	33	31	0.754

*Estimated assuming Hardy–Weinberg equilibrium.

DISCUSSION

The frequency of the NP-H allele in a number of cattle breeds is given in Table 3, together with the calculated values under study. The Gir and Guzerat cattle breeds (*Bos indicus*) could be considered monomorphic for nucleoside phosphorylase H type, since only one animal was found with phenotype NP-L in the Gir breed. These breeds, together with Nellore and Indubrazil, are the only cattle breeds known, up to now, to have such high frequencies for

Table 2. Number of phenotypes for the Canchim cattle by sex and chi-square test for sex phenotype independence

Phenotypes	Males	Females	Total	χ^2
NP-H	136	152	288	3.50 NS*
NP-L	208	267	475	

*NS, not significant, $P > 0.05$.

Table 3. Nucleoside phosphorylase NP-H allele frequency in different breeds analysed

Breeds	Number of animals	Frequency of NP-H allele	Authors
Belgian	585	0.067	1
Campine Red and White	158	0.069	1
Charolais	130	0.256	1
Friesian Black and White	91	0.056	1
German Black Pied (Morocco)	50	0.051	2
German Fleckvieh (Morocco)	46	0.234	2
Blonde Oulmes Zaer (Morocco)	80	0.113	2
Blonde Oulmes Zaer (El Kondia)	115	0.171	2
Brown Atlas (Morocco)	40	0.368	3
Sayaguesa (Spain)	147	0.140	4
Morucha (Spain)	101	0.200	4
Alistana Sanabresa (Spain)	157	0.010	4
Blanca Cacereña (Spain)	62	0.480	4
Asturiana de los Valles (Spain)	127	0280	4
Asturiana de la Montaña (Spain)	106	0.260	4
Taurins (Baoule race)	94	0.389	5
Zebu (Sudan type)	89	0.459	5
Crossbred (European–Zebu) 3/8	70	0.539	6
Crossbred (European–Zebu) 1/2	90	0.516	6
Crossbred (European–Zebu) 5/8	31	0.393	6
Crossbred (European–Zebu) 3/4	30	0.261	6
Canchim	475	0.372	Present study
Guzerat	22	1.00	Present study
Gir	194	0.928	Present study
Nellore	120	0.776	Present study
Indubrazil	33	0.754	Present study

1, Ansay and Hanset (1972); 2, Mahin and Mammad (1982); 3, Briouga *et al.* (1981); 4, González *et al.* (1987); 5, Queval and Bambara (1984); 6, Panepucci (1989).

the NP-H allele. The frequency of 0.372 detected for the NP-H allele in the Canchim breed is very close to the calculated frequency of 0.44 considering the proportions of 5/8 Charolais and 3/8 Zebu (Indubrazil) which constitute this breed. All European breeds and most of the breeds from Spain studied by González *et al.* (1987) have very low values for NP-H, the Charolais and Blanca Cacereña breeds having the highest values, 0.26 and 0.48 respectively.

The use of phosphate buffer and the utilization of the starch gel (either corn or potato starch) in two rows proved to be an extremely cheap methodology for this enzyme, which could easily be used in all laboratories as another genetic marker. It would be very interesting to know the nucleoside phosphorylase frequency in the remaining *Bos indicus* breeds, which could then be used as a significant marker in population studies. The unusually high frequency of NP-H detected in the *Bos indicus* breeds could represent an adaptation to the tropics, as was suggested by Briouga *et al.* (1981) and Queval Bambara (1984). The authors would rather believe that it is a characteristic of the Zebu breeds and that this allele could well be responsible for a selective advantage in certain environments.

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