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Isozyme characterization of cattle (Bos taurus) and American buffalo (Bison bison) cell cultures

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Summary

Four Bovidae cell lines (BEK-1, MDBK, Bu and EBTr) were characterized by means of enzymatic biochemical markers. Out of 15 enzymatic systems, 3 — adenosine deaminase (Ada), phosphoglucomutase (Pgm) and nucleoside phosphorylase (Np) — were found to be polymorphic and quite suitable for biochemical identification of each cell line. The Bu cell line has shown a Np phenotypic pattern which could be distinctive of the *Bison bison* species.

Introduction

Inter- and intraspecific cell-to-cell contamination is of possible occurrence in cell cultures. Detection of such contamination has been made much easier by the introduction of modern karyological, biochemical and immunological procedures (De Oca et al., 1969; O'Brien et al., 1977). The purpose of this paper is to present an isozymic characterization of four Bovidae established or registered cell lines currently used in virological laboratories. Fifteen enzymatic systems were studied. When a polymorphism was discovered it was checked by comparison with cattle *(Bos taurus)* foetal kidney extracts, primary culture of cattle foetal kidney derived from the same animal and buffalo *(Bison bison)* red and white blood cells.

Material and methods

Cell cultures

Continuous or registered cell lines. MDBK cells, EBTr cells and Bu cells were purchased from Flow (Great Britain) and are registred in the American Type Culture Collections (Anon., 1972). BEK-1 cells were kindly furnished by Dr Inaba, from the National

Institute of Animal Health, Kodaira, Tokyo, Japan. The cells were grown in minimum essential medium (MEM) — Earle's BSS (Flow, Great Britain), reduced bicarbonate (850 mg/litre) with non-essential amino-acids (NEAA; Flow, Great Britain), penicillin (100 000 I.U./litre) and streptomycin (100 mg/litre). This medium was supplemented with 10% foetal calf serum in the case of MDBK cell line, with 20% foetal calf serum in the cases of EBTr and Bu cell lines and with 10% foetal calf serum and 10% of tryptose phosphate broth at usual concentrations for the BEK-1 cell line.

Primary cell culture. Kidneys were aseptically removed from a cattle foetus and cut in small pieces which were rinsed in PBS (phosphate buffered saline). Trypsine was then added to obtain primary cells, separated by centrifugation. They were then grown in the same medium as MDBK cells.

Before electrophoretic analysis, the cells were rinsed three times with PBS and stripped off the glass with a rubber policeman. They were suspended in NaCl 0.9% and centrifuged in a Sorvall GLC-1 for 15 minutes at 3000 rev/min; after centrifugation, the supernatant was discarded.

Electrophoresis

One volume of packed cells was suspended in two volumes of phosphate buffer (KH_2PO_4/Na_2HOP_4 0.1 *M*, pH 7.25) and then sonicated for 10 seconds (MSE 150 Watt Ultrasonic Desintegrator). Foetal kidney extract was obtained by homogenization of one volume of organ into three volumes of the same buffer. Preparations of cattle and buffalo haemolysates and leucolysates from whole blood (buffalo blood was kindly provided by Mr van den Bergh, director of the Belgian Zoological Garden of Antwerpen) have been described elsewhere (Ansay & Hanset, 1972a; Widar et al., 1974). The electrophoretic procedure employed either Cellogel-RS strips (Chemetron, Milano, Italy) pre-soaked in Uriel's electrode buffer (Tris 10 m*M*, glycine 333 m*M* pH 7.8) (Uriel, 1966) or polyacrylamide-agar slides (10 cm × 12 cm; cyanogum 41, 3.5%; agar 1%) presoaked in Shows gel buffer (Tris 8 m*M*, citric acid 3 m*M*, pH 6.7). The electrophoresis was conducted at 200 V with Uriel's electrode buffer in the first case and at 400 V with Shows electrode buffer (Tris 8 m*M*, citric acid 86 m*M*, pH 6.3; Shows et al., 1969) in the other case. Enzymatic activity was detected according to usual staining procedures (Shaw & Prasad, 1970; Widar et al. 1975). Particular staining methods used in the study of Ada, Pgm and Np polymorphisms in cattle were previously described by Ansay and co-workers (Ansay & Hanset, 1972b, 1972c; Ansay et al., 1971).

Results

Fifteen enzymatic systems were tested (Table 1). Within the experimental conditions three systems have disclosed electrophoretic variation, eight were non-polymorphic

Table 1. Electrophoretic variability of fifteen enzymatic systems tested on the MDBK, BEK-1, Bu and EBTr Bovidae cell lines.

Enzymatic systems	Results*
Adenosine deaminase (E.C. 3.5.4.4)	+
Adenylate kinase (E.C. 2.7.4.3)	—
Fumarate hydratase (E.C. 4.2.1.2)	no activity
α-Glycerophosphate dehydrogenase (E.C. 1.1.1.8)	no activity
Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49)	—
Glutamate oxaloacetate transaminase (E.C. 2.6.1.1)	—
Glutamate pyruvate transaminase (E.C. 2.6.1.2)	no activity
Glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12)	no activity
Lactate dehydrogenase (E.C. 1.1.1.27)	—
Malate dehydrogenase (E.C. 1.1.1.37)	—
Mannose phosphate isomerase (E.C. 5. 3.1.8)	—
Nucleoside phosphorylase (E.C. 2.4.2.1)	+
Phosphogluconate dehydrogenase (E.C. 1.1.1.44)	—
Phosphoglucomutase (E.C. 2.7.5.1)	+
Phosphoglucose isomerase (E.C. 5.3.1.9)	—

* +: polymorphic systems; —: non-polymorphic systems.

and four were difficult to interpret because of non-existing or too faint enzymatic activity.

Ada zymograms disclosed two different phenotypes called B present in MDBK, BEK-1, Bu and CD present in EBTr by reference to the AC phenotype from the primary

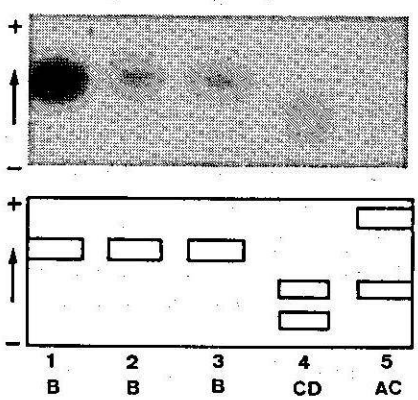

Fig. 1. Ada electrophoretic pattern from Bovidae cell cultures. 1: BEK-1 cell line; 2: MDBK cell line; 3: Bu cell line; 4: EBTr cell line; 5: primary cell culture. Polyacrylamide-agar electrophoresis, 400 V, 45 min.

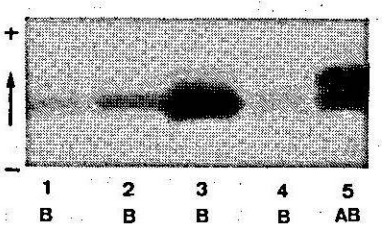

Fig. 2. Pgm electrophoretic pattern from Bovidae cell cultures. 1: primary cell culture; 2: EBTr cell line; 3: Bu cell line; 4: MDBK cell line; 5: BEK-1 cell line. Cellogel-RS electrophoresis, 200 V, 45 min.

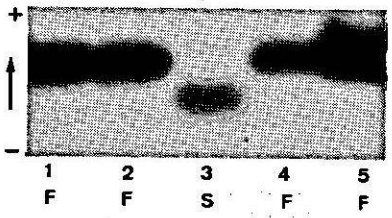

Fig. 3. Np electrophoretic pattern from bovidae cell cultures. 1: primary cell culture; 2: EBTr cell line; 3: Bu cell line; 4: MDBK cell line; 5: BEK-1 cell line. Cellogel-RS electrophoresis, 200 V, 45 min.

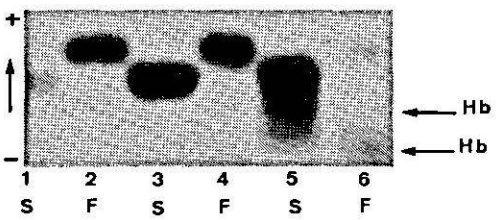

Fig. 4. Comparison of Np zymograms from cattle and buffalo extracts. 1: Bu cell line; 2: MDBK cell line; 3: buffalo white blood cells; 4: cattle white blood cells; 5: buffalo red blood cells; 6: cattle red blood cells. Cellogel-RS electrophoresis, 200 V, 45 min.

cell culture (Fig. 1). The Pgm electropherograms were generally characterized by the single-banded B phenotype except for the BEK-1 line which was of the AB phenotype (Fig. 2). Np zymograms showed two phenotypes: fast (F) and slow (S), the slow moving variant being of buffalo origin (Fig. 3). That this last variant is not a result of cell cultivation per se is suggested by Fig. 4 which shows that buffalo erythrocytes and white cells display the same 'slow' phenotype.

In every case there was a complete identity of the electrophoretic pattern of the primary culture and the foetal cattle kidney extracts derived from the same animal.

Discussion

Established cell lines are now a prominent tool of virological laboratories and differ strongly from the point of view of their susceptibility to various viruses. In veterinary medicine, MDBK cells are very appropriate for the growth of infectious bovine rhinotracheitis virus (bovid herpesvirus-1; Pastoret et al., 1975) and are susceptible to bovine rotavirus (McNulty et al., 1976). BEK-1 cells have been used by Inaba et al. (1976) to cultivate bovine coronavirus. As mixing of cell lines is a rare but possible event, identification criteria are of prime importance.

The main result of this work is to show that the identity of the aforementioned cell lines can be established by biochemical criteria, i.e. the use of enzymatic polymorphisms. Two already described polymorphisms are of particular significance. Ada polymorphism in cattle leucocytes is based on an autosomal four-allele system with ten phenotypes (Ansay & Hanset, 1972b). These four alleles are expressed in our cell cutlures: the BEK-1, MDBK and Bu cell lines are of the B phenotype, the EBTr cell line is of the CD phenotype and the primary cell culture is of the AC phenotype. Allelic variation of the slow moving isozyme of Pgm (Pgm-3) has been described in cattle (Ansay et al., 1971). It is a two allele system with the B allele prevailing (95%) in Belgian breeds. Four

out of the five cell cultures are also of the B phenotype, the BEK-1 cell line being of the AB phenotype. On the other hand, extensive studies of Np in Belgian cattle have disclosed the existence of quantitative and qualitative polymorphisms in red blood cells (Ansay & Hanset, 1972c; Ansay, 1975) while the zymograms obtained with nucleated cells of the same animals were indistinguishable. In this case Np electrophoretic pattern clearly distinguishes the Bu cell line which shows a very distinctive slow migration, most probably species-specific. Unfortunately we were unable to extent our study to cover a great number of buffalo. Eventually, however, this new phenotype could be a useful marker of the *Bison bison* species (Stormont et al., 1977).

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