

CHROMOSOME ANALYSIS OF THE IBRS-2 SWINE CELL LINE BY BANDING METHODS

E. Muñoz and N. Brum-Zorrilla

ABSTRACT

A karyotypic study was performed on the stable swine cell line IB-RS2 cl. 17. A modal number of 37 chromosomes was found and G-banding permitted the characterization of all chromosomes. C bands were located in the pericentromeric regions, while the NORs were visualized in the chromosomes carrying a secondary constriction (G II and G IV-4). The sex chromosomes were also identified.

INTRODUCTION

Since cell lines undergo changes over successive cultures, periodic chromosome checks permit the detection of any possible alterations. At the Cytoculture Division (DILFA) of the Department of Agriculture and Fishing of Uruguay, studies are being conducted on the foot-and-mouth disease virus which causes heavy economic losses to the cattle industry in the country. The most frequently used cell line in these studies is IBRS-2, which originated from a primary culture of the cortex of a 3-month-old female swine kidney at the Biological Institute of São Paulo, Brazil.

In view of the importance of this line, we undertook a cytogenetic study to check its chromosomes. Isolated and stabilized clones were selected for sensitivity and resistance to foot-and-mouth diseases virus. A chromosome analysis of this line was initially done by Castro and Pisani (1964) and Castro (1970), who classified the elements but had difficulty in determining the members of one group.

In the present investigation we focused on the identification of the chromo-

some belonging to that group, bearing in mind Castro's (1970) proposition that the genes regulating resistance to foot-and-mouth disease virus may be located there. G-, C- and Ag-NOR staining techniques were used for better chromosome identification.

MATERIAL AND METHODS

The IBRS-2 swine cell line clone 17 between passage 76 and 97 was used for the present study. This cell culture is being maintained in Eagle nutrient medium modified according to Macpherson and Stoker (1962) and supplemented with 10% fetal calf serum. After 48 hours of incubation, cells were treated with saline trypsin versene and medium containing final colchicine concentration of 0.5 $\mu\text{g/ml}$. After four hours of incubation, the medium was replaced and hypotonic treatment with 0.075 M KCl was carried out. The hypotonic solution was removed 25 minutes later and cells were fixed in 3:1 methanol:acetic acid. G bands were induced by the trypsin technique of Seabright (1971). C and NOR bandings were obtained by the procedure of Ruffas *et al.* (1983). Chromosomes were classified as proposed by Ruddle (1961) and modified by Castro and Pisani (1964).

RESULTS

The karyotype was constructed by examining 30 selected cells. Chromosome analysis showed a modal number of 37 elements. (FN = 62), but variation from 36 to 38 chromosomes was also detected. In the metaphases with 38 chromosomes, the additional chromosome was a small microchromosome (Figure 1A). Metaphases with 36 chromosomes lacked this microchromosome and other elements.

G-banding

G-banding permitted the identification of all chromosomes (Figure 1B). Examination of the patterns revealed that: 1) the long arm of chromosome G III-2 had a band distribution similar to that of one of the elements in group G VIII-1, denoted "false trisomy"; 2) the marker chromosome M I had the same band sequence as the long arm of G III-1, but distribution differed between arms; 3) the banding pattern of the long arm of chromosome SA-1 was similar to that of the acrocentric chromosome G VI.

C-banding

Heterochromatin was detected in the centromeric regions of some elements. A large heterochromatin block appeared in the submetacentric chromosome of group

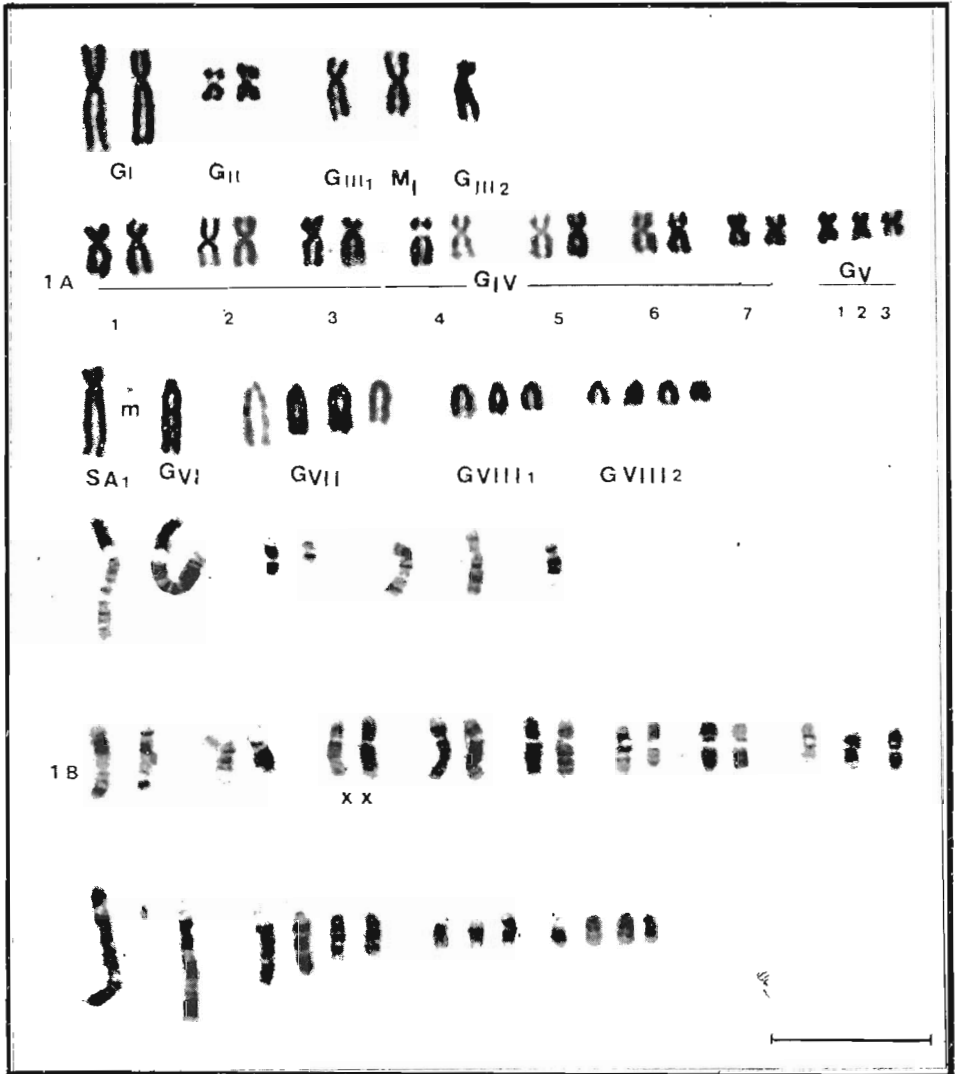


Figure 1 - A, Giemsa stained karyotype of the IBRS-2 swine cell line. Letters and numbers indicate different chromosome groups: G_I , G_{II} , G_{IV} , G_V , G_{VI} , G_{VII} , G_{VIII1} , and G_{VIII2} . They also show the line marker chromosomes G_{III-1} and M_I , C_{III-2} , S_{A1} . The letter m indicates the microchromosome. B, G-banding karyotype of the IBRS-2 cell line. Chromosomes are ordered according to the classification indicated in Figure 1A.

G_I and in the acrocentric chromosomes of groups S_{A-1} , G_{VI} , G_{VII} , G_{VIII-1} and G_{VIII-2} . Little or no heterochromatin was observed in the other elements (Figure 2A).

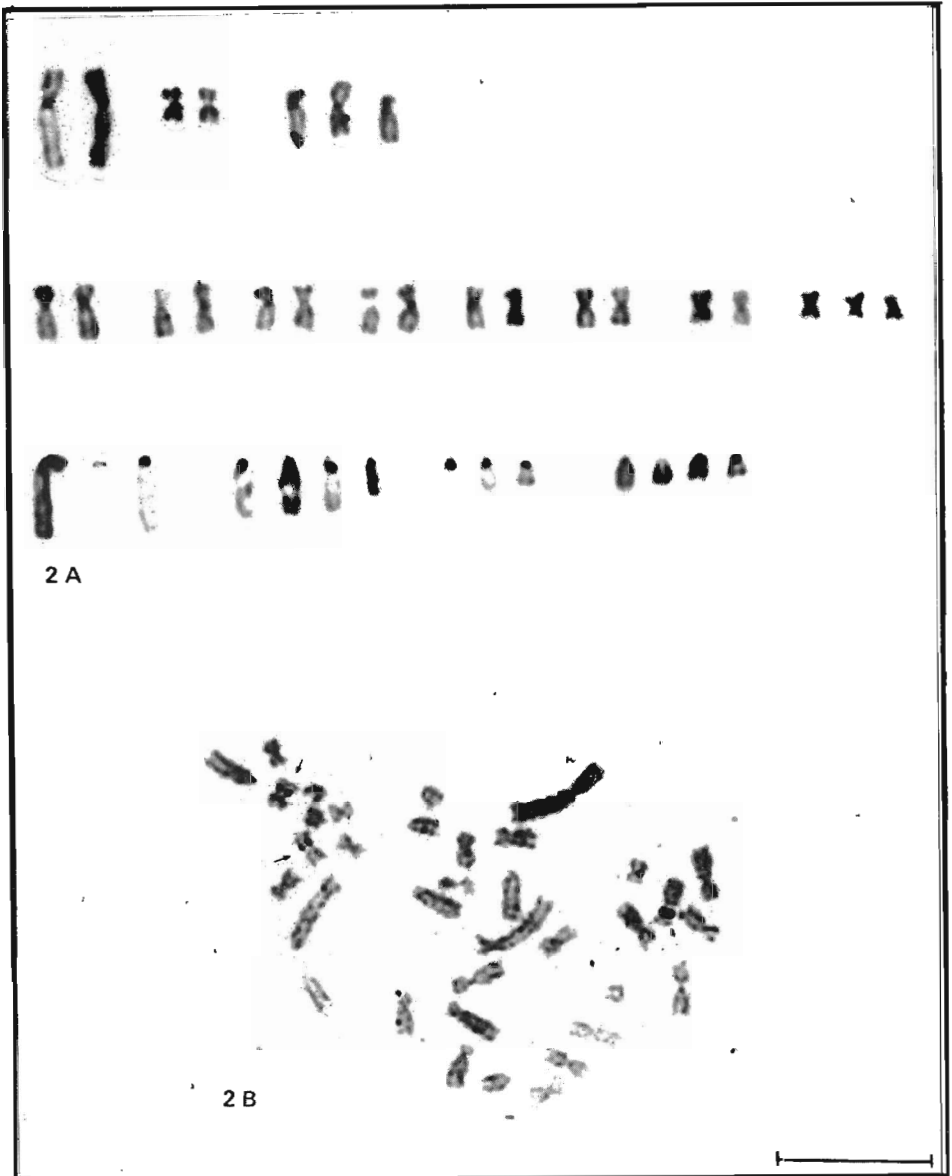


Figure 2 - A, C-banded karyotype. B, Metaphases of IBRS-2 after NOR staining. Three chromosomes show positive NOR's (arrows). Scale bar in all figures represents 10 μm .

Chromosome with NORs were observed. Two positive Ag dots were located near the constriction present in both chromosomes of Group G II and other NORs were observed in the centromeric regions of one homologue in group G IV-4 (Figure 2B).

DISCUSSION

Among several clones isolated from the parental culture of swine cells, we used IBRS-2 classified according to Castro (1970) as cell line clone 17. In general our results agree with those of Castro and Pisani (1964) and Castro (1970), except that we were able to identify all chromosomes. The sex chromosomes of the cell line were identified by comparing our G-banding pattern with the X chromosome patterns obtained by Hanzen-Melander and Melander (1974), Pace *et al.* (1975) and Hanzen (1980) for the pig. Comparison with their findings enabled us to place the X chromosome in the G IV-3 group.

Some chromosome aberrations which were perpetuated through cell passages were used to trace the genealogy of cell clones by Castro (1970). According to Castro's study, an inversion appeared to have occurred in chromosome G III-1, leading to the formation of the M I chromosomes. However, G-banding ruled out the possibility of a pericentric inversion or of isochromosome formation. This author also noted the absence of one G III-2 chromosome, but a compensation appeared as an apparent "trisomy" among the members of G VIII-1. In our material, the long arm of G III-2 showed banding similar to that of an acrocentric in the G VIII-1 group. Finally, the fragment produced during the rupture of the short arm of G III-2 may have contributed to the formation of either the microchromosome or the short arm of S A-1.

We observed variable heterochromatin distribution and amount in the IBRS-2 cell line. Only the large submetacentric G I chromosome showed a dark prominent band proximal to the centromere region, while the acrocentric chromosomes always showed heterochromatin blocks near this region. Therefore, C-banding cannot be used for chromosome identification in this cell line.

The NOR distribution pattern observed here was identical to that reported by Czaker and Mayr (1982) and by Mayr *et al.* (1984) for *Sus scrofa domestica*. We compared our results with those obtained by these authors and found that NOR regions are located on two chromosome pairs with secondary constrictions.

It has been postulated that, in swine cell lines PK 15-3 and IBRS-2 the genes that control susceptibility to foot-and-mouth disease virus may be located on chromosomes G VI and G IV (Castro, 1970; Koseki and Abuhab, 1972).

Since we now know with more certainty the chromosome composition of the cell line used to detect susceptibility to foot-and-mouth disease virus, we believe we can complete these studies and obtain a better understanding of virus-chromosome interaction using a new methodology.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Francisco Salzano for his valuable suggestions and review of the manuscript.

We also thank Br. A.M. Cazenave for technical assistance and Mrs. Nelly Pérez for typing of manuscript.

RESUMO

Foi realizado estudo cariotípico da linhagem celular suína estável IB-RS2 cl. 17. Encontrou-se um número modal igual a 37 cromossomos e a técnica de bandeamento G permitiu a caracterização de todos os cromossomos. As bandas C estavam localizadas nas regiões pericentroméricas, enquanto que as NORs foram visualizadas em cromossomos portadores de constrição secundária (G II e G IV-4). Os cromossomos sexuais foram também identificados.

REFERENCES

- Castro, M.P. (1970). Clonal variation in the swine kidney cell line IBRS-2 in relation to morphology, karyotype and susceptibility to the foot-and-mouth disease virus (F.M.D.V.). *Arq. Inst. Biol. São Paulo* 37: 103-127.
- Castro, M.P. and Pisani, R.C.B. (1964). Estudos sobre o complemento cromossômico de linhagem celular suína IBRS-2 suscetível ao vírus aftoso. *Arq. Inst. Biol. São Paulo* 31: 155-166.
- Czaker, R. and Mayr, B. (1982). Comparative studies on the polymorphism of nucleolus organizer (NORS) in four breeds of domestic pig (*Sus scrofa domestica*) with special emphasis on the development of breeds. *Z.F. Zool. Syst. Evol.* 20: 233-241.
- Hanzen, K.M. (1980). Identification of the X chromosome of domestic pig (*Sus scrofa domestica*). *Ann. Genet. Sél. Anim.* 12: 225-232.
- Hanzen-Melander, G. and Melander, Y. (1974). The karyotype of the pig. *Hereditas* 77: 149-158.
- Koseki, I. and Abuhab, T.G. (1972). Virus da febre aftosa em clones celulares de linhagem PK-15-3. II. Correlação entre cariótipo e suscetibilidade. *Arq. Inst. Biol. São Paulo* 39: 291-309.
- MacPherson, I. and Stoker, M. (1962). Polyoma transformation of hamster cell clones, and investigation of genetic factors affecting cell competence. *Virology* 1: 147-151.
- Mayr, B., Schweizer, D. and Geber, G. (1984). NOR activity, heterochromatin differentiation and the Robertsonian polymorphism in *Sus scrofa*. *J. Hered.* 75: 79-80.
- Pace, J.W., Srivastar, P.K. and Lasley, J.P. (1975). G-band patterns of swine chromosomes. *J. Hered.* 66: 344-348.
- Ruddle, P.H. (1961). Chromosome variation in cell populations derived from pig kidney. *Cancer Res.* 21: 885-894.
- Ruffas, J.S., Gosálvez, L., López-Fernández, C. and Cardoso, H. (1983). Complete dependence between AgNORs and C-positive heterochromatin revealed by simultaneous Ag NOR C-banding method. *Cell Biol. Int. Rep.* 7: 275-281.
- Seabright, M. (1971). A rapid banding technique for human chromosomes. *Lancet* 2: 971-972.