SPECIFIC VARIABILITY AND SOME PECULIARITIES OF M13 PIG DNA MINISATELLITES

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SUMMARY

The interbreed pig DNA peculiarities of durok, landras and large white pigs were analised with the help of the DNA-fingerprint technique. Certain interbreed differences in pigs' DNA-fingerprints have been found.

INTRODUCTION

The problem of genetic variability is one of the central in agricultural animals genetics. The creation of a great number of highly specialized breeds is closely connected with it. Nowadays a lot of various genetic variability study methods exist. Immunologic typing methods using various antigenes are the most widely spread. They are the polymorphism detection methods, dealing both with separate proteins possessing the known biochemical function and with many individual proteins (protein fingerprint). Receiving of additional information for genetic variability studies on the polynucleotide DNA sequence level is available due to the restriction analysis method application.

Sus scrofa interbreed variavility study on the DNA level makes it possible to judge about interbreed relations, phylogenetic links as well as breed formation mechanisms.

The interbreed DNA differences are easily detected at more complete genome comparison, which is achieved by polylocus molecular-genetic markers application. One of these moleculargenetic marker's system is the tandem repetition number polymorphism of M13 minisatellites.

MATERIALS AND METHODS

Landras, durok and large white pigs have been used for the experiments.

DNA was extracted from the boar sperm and digested by MspI. The electrophoresis and the transfer to Kapron membranes as well as the hybridization were conducted in the conditions described by(Westneat et al., 1988). The labelled probe preparation peculiarity lied in the usage of the hybridization primer which hybridization in the direct proximity to M13 phage repetitions, rich in guanine, is followed by the matrix and probe desintegration.

RESULTS

The radioauthographs analysis showed that the basic number of the information bands is located in the area from 4 to 9 Kb. The mean

number of bands per animal equals 17.86±0.79. The minimum number of bands is 15, and the maximum is 21. The mean probability of bands equals 0.541±0.551. Common for the three breeds were 2 bands sizing about 4.8, 5.4 Kb. Specific for durok pigs were three fragments sizing about 4.0, 4.6 and 7.1 Kb, and those for landras pigs 5.5, 9.5 and 10.0 Kb. No specific bands have been detected for the large white breed.

The M13 minisatellite pig's DNA peculiarity is the different locuses' fragments superposition in a phoregram; DNA digested by HinfI gives out two bands of 4.6 and 6.1 Kb, and DNA digested by AluI gives out two low-molecular bands.

DISCUSSION

We studied the M13 mst pig DNA individual peculiarities with the purpose of determining the coincidence of the pig DNA hybridization pictures probability, labelled M13 phage DNA being used. The hybridization pictures individual peculiarities were detected for each breed pig DNA according to the number and location of their bands. It's worth mentioning, that the basic number of dehybridizing information fragments was located within the range of 4-10 Kb. Fragments sizing less than 4 Kb haven't been analysed. We afford certain subjectivity in radio-authographs evaluation for the reason of uneven band intensiveness.

Some landras pig DNA samples possess the maximum number of hybridizing fragments within the above-mentioned range of 21. Some large white and durok pig DNA samples possess the minimal number of 15. MspI restrictaze application gives the possibility to receive mean 17.6±0.805 bands per animal. The bands frequency ranged from 1 to 0.09. The mean band probability for unrelated large white pigs equalled 0.508±0.039. The evaluation of all fragments coincidence probability in two animals has been counted according to the method described by (Barysheva et al.,1989). In our research this estimation for unrelated large white pigs equalled to 0.9999947.

With the purpose of the breed, types and lines passportization possibility study we have conducted M13 mst DNA hybridization pictures comparison of durok and landras pigs.

Various pig breed DNA was characterized both by individual peculiarities and by definite bands common with all animals under study. These bands probably refer to the category of genetic markers which are the characterizing indications of the Sus scropha species. Bands specific for the species were used as a sort of a marker for comparing the results received at different radio-autographs in addition to the molecular weight markers.

The comparative analysis of the pig DNA hybridization pictures for landras and durok breeds showed the common picture type both for the mean bands number of 17.86±0.79 per animal and for the hybridization fragments location in the area from 4 to 10 Kb.

However, different pig breed DNA possess some peculiar bands. They are probable to be referred to the genetic markers characterizing the breed. For durok pigs they are hybridization fragments of

approximately 4.0, 4.6 and 7.1 Kb. For landras pigs the specific hybridization fragments sized approximately 5.5, 9.5 and 10.0 Kb.

The breed-specific pig DNA hybridization bands existance with landras and durok pigs is probably connected with the "bottle neck" effect, which takes place at pigs' delivery to the territory of the former USSR.

The large white breed making about 90% of all pigs in Ukraine possesses much more expressed polymorphism of M13 mst DNA. However, at pig genetic marking it seams necessary not to operate by the breed specific bands, but to do as in the case of biochemical markers (allelic frequencies).

The molecular base of mst DNA family members polimorphism lies in the different number of repeated units in each mst locus. To detect this type of polimorphism it's necessary to restrict DNA by the restriction enzyme, which cuts frequently enough, but doesn't destroy the mst locuses integrity. In the given paper we studied restrictases Alu I, BsuR I, Hinf I, Msp I, Sau3AI and Tag I. The necessity of studying such a large enzyme spectrum lied in the fact that sometimes there occur restriction DNA fragments similar in size. It's impossible to desintegrate these fragments by means of electroforesis, therefore we evaluated this or that restrictase's fittness according to its ability to create the maximum number of bands in the radio-authographer.

We've determined certain peculiarities of pig M13 mst DNA. Thus, at restriction by the Alu I and Hinf I ensymes, two intensive bands for each ensyme were seen on the background of a small number of bands: 5-8. For Hinf I these were bands sizing 4.6 and 6.1 Kb, and for Alu I they were two bands sizing less than 2 Kb.

Creation of such units of restriction frasments is, probably, the peculiarity of M13 mst pig DNA, which is likely to possess restriction sites in the middle of the mst sequences.

REFERENCES

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