

MICROSATELLITES ANALISIS FOR THE STUDY OF BIODIVERSITY AND TRACEABILITY OF THE PIG

ANALIZA MICROSATELITILOR PENTRU STUDIUL BIODIVERSITATII SI TRASABILITATII LA SUINE

IUDITH IPATE¹, G.BREM², A.T.BOGDAN¹, MONIKA GUTSCHER², I.
SEREGI³, G. F. TOBA², L. ZOLDAG³, A. MAROTI-AGOTS³

¹Romanian Academy- CSCBA

²Faculty of Veterinary Science Viena

³Faculty of Veterinary Science Budapesta

Traceability of animals and animal products has a priority for governments of the European countries. The great development reached by the molecular genetic in last decades, has determined a high knowledge of the genome of the different species. The Deoxyribonucleic Acid (DNA) of each animal is different (with the exceptions of monozygotic twins and clones). Since the genome of each animal contains approximately three billion DNA units, the range for variation among the DNA sequences of animals is enormous, consequently DNA markers analysis allows assuring a traceability of 100% in the meat industry. A methodology using 17 ISAG (International Society for Animal Genetics) DNA microsatellite markers is proposed for meat traceability. Principal methods used to reveal DNA polymorphism are described as their applicability in species identification and meat traceability. The objective it was analysis the DNA profile of pig with 10 microsatellite markers and results of meat identity control.

Keywords: genetic diversity, microsatellites

Introduction

The analytical methods used for species identification and authenticity of foods rely mainly on protein and DNA analysis. The protein-based methods include immunological assays electrophoretical and chromatographic techniques. More recently, DNA molecules have been the target compounds for species identification due to the high stability compared with the proteins, and also to their presence in most biological tissues, making them the molecules of choice for differentiation and identification of components in foods, and a good alternative to protein analysis. Most DNA-based methods for species identification in foods consist on the highly specific amplification of one or more DNA fragments by means of polymerase chain reaction (PCR). DNA microsatellite markers are proposed for meat traceability.

Materials and Methods

Tissue samples were collected from pigs of the ROMSIUNTEST- Peris - Ilfov and SCDB-Rusetu station at Buzau using Typifix system.

-10 microsatellites were amplified in multiplex reactions and analysed on ABI310 genetic analyser.

- The probes it works in Agrobiogen Laboratory at the Vienna.

Tissue collection with TypiFix™ –System

The TypiFix™ ear tag system is a combination of a conventional ear tag with a simultaneous tissue sampling technology. By ear tagging the farm animals, the tissue samples are automatically collected and sealed in the TypiFix™ sample containers, where the tissue samples are preserved at ambient temperature and can be used for protein or DNA based assays. The easy handling of the TypiFix™ ear tag system allows economic sampling of whole populations and is therefore an effective tool for analysis of genetic markers for paternity control, traceability and breeding traits. The Typi-Fix®-System is a procedure for the collection of DNA containing tissue samples avoiding all these hurdles and problems. With the Typi-Fix®-ear tags the animal is marked - in the usual convention - with a plastic ear tag. At the same time, however, a tissue sample is taken by the spike of the ear tag which immediately after the collection is packaged in a special plastic container (sample receiving container) labelled with the (bar coded) animals ear tag number. After collection the preservation and preparation of the DNA is initiated automatically by substances which are held in stock in the sample receiving container. The identification number of the samples can be registered by a reading device (scanner). The sample container is connected to the eartag by a plug and socket and is easily removed after the eartag has been affixed and the tissue sample simultaneously collected. If desired, the sample container can also be used without the eartag. After pigs tissue collection with ear tagging, we collected meat probes in abattoir.

- The porcine agreed microsatellite markers use for:

- Set I is: S0005 for chromosome 5 and range 205-248, S0090 for chromosome 12 and range 244-251, S0155 for chromosome 1 and range 150-166, SW857 for chromosome 14 and range 144-160, SW240 for chromosome 2 and range 96-115
- Set II is: SW24 for chromosome 17 and range 96-121, SW951 for chromosome 10 and range 125-133,

DNA purification with DNA FIX columns an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix™ samples. The sorbents retain protein and other contaminants, while the DNA passes the column in the exclusion volume.

DNA isolation and purification can be automated through the use of a pipetting robot and a special one-step procedure (Nexttec technology). PCR reactions with these samples can also be prepared automatically. The results of the multiplex PCR

analyses are linked with the scanned identification number and saved in the animal data bank.

Gel electrophoresis of NCC™ purified DNA from 88 TypiFix™ eartag samples : 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/EtBr gel. The DNA concentration is about 10 ng/µl or greater = negative control

Results and Discussion

The results of DNA profile of pig obtained by analysis of 10 microsatellite markers and results of identity control is presented in the next tables.

The meat of each animal it was identifications with the microsatellite markers. The analysis based on microsatellite data has demonstrated the possibility to genetically characterize the breeds studied and to distinguish the origin of animals.

The hybrid of Peris and the hybrid of Rusetu has the in scheme of the origin common parts. This part is demonstrated with the results of the microsatellite analysis.

Each animal have illustrated the carcasses identification with this methods. These systems can also be used other large-scale for animals products identification with DNA analysis.

Table 1

Lab no.	Typifix no.	Animal ID	Sample	
US080007	1	1	animal	Peris
US080008	51	51	animal	Peris
US080014	57	57	meat	Peris
US080009	52	52	animal	Peris
US080015	58	58	meat	Peris
US080010	53	53	animal	Peris
US080017	60	60	meat	Peris
US080011	54	54	animal	Peris
US080016	59	59	meat	Peris
US080012	55	55	animal	Peris
US080018	61	61	meat	Peris
US080013	56	56	animal	Peris
US080019	62	62	meat	Peris
US080039	94	RO3072556672	animal	Rusetu
US080040	95	RO3074328144	animal	Rusetu
US080041	96	RO3072977509	animal	Rusetu

DNA profile of pig samples obtained by analysis of 10 microsatellite markers and results of identity control

Table 2

S0005		S0090		S0101		S0155		S0355	
231	235	242		211		158	160	245	273
239		242	246	211		160	162	245	
239		242	246	211		160	162	245	
239		242	246	195	213	156	158	245	259
239		242	246	195	213	156	158	245	259
201	219	244	250	211	213	160	162	245	249
201	219	244	250	211	213	160	162	245	249
227	239	242		209	211	156	158	245	249
227	239	242		209	211	156	158	245	249
215	229	240	246	209		158	160	245	273
215	229	240	246	209		158	160	245	273
219	239	242	246	209	213	148	158	245	259
239	245	240	250	209	211	160		245	249
219	245	240	244	207	209	158	160	245	259
221	245	244	246	195	209	158	160	245	259

DNA profile of pig obtained by analysis of 10 microsatellite markers and results of identity control

Table 3

S0386		SW24		SW240		SW857		SW951	
174	176	101	107	93	105	143	149	120	
174		107	113	95	111	147	149	120	122
174		107	113	95	111	147	149	120	122
174		93	101	91	107	139	151	120	
174		93	101	91	107	139	151	120	
174		93	101	93	107	139	149	120	
174		93	101	93	107	139	149	120	
174	176	99	117	95		149	151	120	122
174	176	99	117	95		149	151	120	122
174	176	113		95		139	149	120	
		113		95		139	149	120	
		101		93	95	147	155	120	
174	176	107	113	91	111	139	143	120	
176	184	93	107	95	105	149		120	
168	176	113		95	105	149		128	

The method based on microsatellite markers gives concrete results and is a valuable tool for the meat industry. The applicability of the methods is very important because give the transparency needs of the market in very short time.

Conclusion

1. The TypiFix™ ear tag system is simple, one-step collection and preservation of tissue samples
2. The TypiFix™ ear tag system is fast, fully-automated and economical preparation of DNA
3. This method is to be performed much more quickly and economically than is currently possible with the traditional methods of sample preparation.
4. The analysis performed with the panel of the 10 markers still able to give a correct result for all pigs and the identification the meat products it was 100%.

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