COMMUNICATION

A simple DNA based method for determination of pure Black Slavonian pigs

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ABSTRACT - The aim of this work was to determine the MC1R genotype of Black Slavonian pigs and to find an efficient and simple PCR-RFLP method, based on differences in MC1R genotype, to distinguish between purebred Black Slavonian pigs and their crossings with commercial pig breeds and Wild Boars. Sequencing of the MC1R exon was performed to determine the genotype of MC1R in Black Slavonian pig breed, which was shown to be MC1R*2. Digestion reactions of both PCR products representing the majority of MC1R exon revealed presence of the BspHII restriction site at position 121 and absence of the AccII and CrfI restriction site at position 240, which is characteristic for the MC1R*2 genotype. A simple PCR-RFLP method, based on different coat colour MC1R gene genotypes was determined by which it is possible to detect potential crossings of autochthonous Black Slavonian pig with commercial pig breeds and also with Wild Boars.

Key words: Black Slavonian pig, Crossings detection, MC1R genotype.

Introduction – Black Slavonian pig breed is critically endangered autochthonous pig breed in Croatia. It was established in the 19th century in the region of Eastern Croatia, near Osijek, by crossing local Mangalitsa pigs with the Berkshire pig breed. Black Slavonian pig breed has been raised in extensive (pastures, woods) and half-extensive conditions (pens with some free space). Extensive keeping provided opportunity for the contact between this pig breed and local Wild Boar population, and also for uncontrolled crosses between Black Slavonian pigs and commercial pig breeds. Phenotypic distinguishing between purebred and F1 crossbred pigs is not possible because of dominant black color of Black Slavonian pig.

Extension (E) is one of the coat color loci, which encodes the melanocortin receptor 1 (MC1R) expressed in melanocytes (Robbins et al., 1993). MC1R signaling determines whether the melanocyte produces black eumelanin or red/yellow pheomelanin. Extension / MC1R is also one of the major coat color loci in pigs and a series of alleles with phenotypic effects has been revealed by sequence analysis (Kijas et al., 2001; Giuffra et al., 2000). Wild
boars possess wild-type alleles; $MC1R^*1$ (European) or $MC1R^*5$ (Japanese wild boar). Two different alleles for dominant black color were detected. Large Black and Meishan pigs carry $MC1R^*2$, while Hampshire possesses $MC1R^*3$. The recessive red coat color of swine is characteristic for Duroc and is associated with $MC1R^*4$. The sixth allele, $MC1R^*6$ was determined in Yorkshire, Landrace and black-spotted pig breeds (Kijas et al., 2001).

The aim of this work was to determine the $MC1R$ genotype of Black Slavonian pigs and to find an efficient and simple PCR-RFLP method, based on differences in $MC1R$ genotype, to distinguish between purebred Black Slavonian pigs and their crossings with commercial pig breeds and Wild Boars.

**Material and methods** – Genomic DNA was isolated from blood samples of 20 Black Slavonian pigs by salting-out method. Two primer pairs were used to amplify the majority of the single exon of $MC1R$ gene. The first pair of primers, MERL1 and EPIG2, was used to amplify a 428-bp product from the 5’ half of the exon, whereas EPIG1 and EPIG3 amplified a 405-bp product from the 3’ half (Kijas et al., 1998). PCR was performed and PCR products from five animals were isolated from agarose gel and sequenced (Macrogen Inc., Korea). 10μl of the PCR-products from all 20 animals were digested by restriction endonucleases $Bsp$HI and $Acc$II. The PCR product MERL1/EPIG2 was digested by $Bsp$HI to determine the genotype at position 121. The genotype at position 240 was determined by $Acc$II digestion of the EPIG1/EPIG3 PCR product, and also by digestion with $Crf$I restriction enzyme. The digestion-reactions were checked on 3% agarose gel.

**Results and conclusions** – Sequencing of both (MERL1/EPIG2 and EPIG1/EPIG3) PCR products from five different animals was performed to determine the genotype of $MC1R$ in Black Slavonian pig breed. The genotype was the same as in the first group of black-coloured pig breeds like Large Black and Meishan (allele $MC1R^*2$). This genotype differs from the wild-type genotype ($MC1R^*1$), and also from the genotypes of commercial pig breeds, raised in Slavonia (eastern Croatia). Those are Large White and Yorkshire with allele $MC1R^*3$, Pietrain with allele $MC1R^*6$ and Duroc with allele $MC1R^*4$ (Figure1).

**Table 1.** Mutations in the pig MC1R gene defining different allelic variants. Codon positions are numbered according to the human sequence. The polymorphic BspHI (TCATGA), AccII (CGCG) and CrfI (GGCC) recognition sites are indicated.

| Pig Breed          | BspHI | AccII | CrfI |
|--------------------|-------|-------|------|
| Wild Boar $MC1R^*1$| GTG   | CTG   | AAT  |
| Black Slavonian $MC1R^*2$ | ATG   | CCG   | AAC  |
| Large White $MC1R^*3$ | GTG   | CTT   | ATC  |
| Pietrain $MC1R^*6$ | GTG   | CTT   | ATC  | AAT  | AAC  | GGC  | GGC  | GGC  | GGC  |
| Duroc $MC1R^*4$   | GTG   | CTT   | ATC  | AAT  | AAC  | GTC  | GGC  | ACI  | GCC  |
|                    | **    | ***   | **   | ***  | **   | ***  | ***  | *    | ***  |
Digestion reactions of both PCR-products revealed presence of the \textit{Bsp}HI restriction site at position 121 and absence of the \textit{Acc}II restriction site at position 240, which is characteristic for the \textit{MC1R}*2 genotype.

By restriction digestion of the MERL1/EPIG2 PCR product with \textit{Bsp}HI restriction enzyme it is possible to detect all potential crossings of Black Slavonian pigs with white coloured pig breeds like Landrace and Yorkshire (\textit{MC1R}*3 genotype) and Pietrain (\textit{MC1R}*6) which don’t possess the \textit{Bsp}HI restriction site at position 121. All potential crossings with \textit{MC1R}*1 (Wild Boar), \textit{MC1R}*3 (Large White, Yorkshire) and \textit{MC1R}*6 (Pietrain) could be detected by restriction of the EPIG1/EPIG3 PCR product by \textit{Acc}II restriction endonuclease, because only Black Slavonian pigs don’t include an \textit{Acc}II restriction site at position 240. By those already described restriction digestion methods it is impossible to eliminate crossings with Duroc, so we searched the sequence for another restriction endonuclease which could distinguish between genotypes \textit{MC1R}*2 and \textit{MC1R}*4.

The \textit{Crf}I restriction site at position 240 was found, which could not only detect crossings with Duroc breed, but also crossings with other commercial breeds and Wild Boars. Namely, Black Slavonian pig breed is the only one without the \textit{Crf}I restriction site at position 240.

With this work, we were able to identify a simple PCR-RFLP method, based on different coat colour \textit{MC1R} gene genotypes, by which it is possible to detect potential crossings of autochthonous Black Slavonian pig with commercial pig breeds and also with Wild Boars.

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