Development of skeletal muscle transcriptome analysis to study pig meat quality

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INTRODUCTION

Control of meat quality is a major goal for porcine production. Meat quality is complex and strongly depends of various criteria (fibres characteristics, intramuscular fat content, type of collagen, proteolysis intensity,...) affected by genetic, nutritional or environmental regulation factors. Until now, experiments were designed to study the expression and the function of a limited number of known or "candidates" genes (myogenic or adipogenic differentiation factors, myosins, hormonal receptors, enzymes, transport proteins...) which could not explain all the complexity. In pig, if major genes have already been identified for two defaults of meat quality (Pale Soft and Exsudative meat and acid meat), most problems are still poorly understood. For example, genes controlling intramuscular fat (imf) content or playing a key role in the appearance of ham destructuration are not known. However, imf content, an important factor for organoleptic quality of meat (taste, flavour, juiciness), is on average 1.5% in the Longissimus Dorsi (LD) muscle, while a study of consumers jury gives an optimal organoleptic quality for a rate of 2.5 to 3% (FERNANDEZ et al., 1999). That is why it is so important today to better manage the imf content without affecting the deposit of external lipids. In the same way, 17% of cooked ham are destructurated and this default causes severe economic losses. The destructuration default was only apparent in fresh deboned hams. Destructurated muscles have then lost their fibrous aspect and formed a soft muscular mass without any organised structure. This change would get closer to PSE meat phenotype (MINVIELLE et al., 2001).

By supplying a profile of a large number of expressed genes, transcriptome analysis will give an access to nearly all the genes which are simultaneously involved and regulated during different biological processes. In order to improve...
livestock management, INRA has set up a large scale analysis of the genome of farm animals which is called AGENAE (HATEY et al., 2000). This new approach will enable the identification of new genes involved in muscle characteristics and meat quality and will provide an explanation for the mechanism of regulation. The results could be used to improve animal breeding and to develop new molecular markers for meat quality.

The studies presented here show preliminary results obtained with the first set of porcine high density macroarray (AGENAE) and the development of this kind of analysis.

METHODS

Animals

Study of intramuscular fat content: Castrated F2 Duroc X Large White pigs were reared at INRA the Magneraud and slaughtered at an average weight of 106 kg. After intramuscular fat content determination in the LD muscle (FOLCH et al., 1957), 2 groups of pigs were formed: low fat content pigs with an average imf content of 1.99% (LF, n=6) or high fat content pigs with an average imf content of 3.43% (HF, n=6).

Study of destructured meat: F2 pigs divergent for meat quality characteristics (France Hybride) were assigned in two groups according to their destruction score (CIELab system): control pigs (C, score=0, n=3) and destructurated pigs (D, score=4, n=3).

Macroarrays

Two kinds of macroarrays have been used. One containing 1056 cDNA from a normalised multi-tissues pig library (AGENAE) and the other containing 1152 cDNA from a normalised skeletal muscle pig library (SOARES, USA). Bacterial clones have been spotted on nylon membranes with a Biogrid spotter (Génopole Toulouse, France).

Hybridisation, image analysis and data extraction

Methods developed here are an adaptation of the Multiplex Messenger Assay (BERNARD et al., 2000). Total RNA were first extracted, their quality was checked and they were pooled in equimolar proportion for all the four groups (LF, HF, C, D). These RNA pools (15 µg) were labelled during the reverse transcription and different kinds of hybridisation conditions were tested. After exposition and scanning (STORM phosphorimager), each spot intensity was quantified using the Imagene software (Biodiscovery) and normalised.
Results and discussion

Each hybridisation gives an image with spots of variable intensities (figure 1).

Figure 1
Hybridisation of multi-tissues (AGENAE) or skeletal muscle macroarray with a cDNA complex probe. The probe was synthesised from skeletal muscle pig RNA.

During labelling, each mRNA is reverse transcribed and its abundance in the probe is proportional to the gene expression level. This expression level corresponds to the spot intensity which is quantified during image analysis with a specific software. Each signal is due to the matching of homologous sequences between the probe and the cDNAs fixed on the membrane (hybridisation process). These spots could change according to the experimental samples. The comparison of gene expression levels between two experiments is done by measurement of the ratio of spots intensities obtained with the two probes. However, there are some technical drawbacks like bacterial growth differences between macroarrays, unequal synthesis of probes and differences between hybridisation conditions which disturbed data analysis. To this end, normalisation of the spots intensities is performed (Bernard et al., 2000).

The analysis of the results is in progress.

Conclusion

These comparative analyses show specific and characteristic expression pattern for our different samples: low fat muscle (LF), high fat muscle (HF), undestructurated ham (C) or destructurated ham (D). For each gene, the level of expression will be known in every condition, it can then be identified as an interesting gene or not. So, this new tool appears well suited and very successful for the study of genes involved in the determinism of pig meat quality.
REFERENCES


