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Search of candidate genes for salting loss of ham by using Fluoro Differential Display (FDD)

V. Russo, D. Bigi, P. Zambonelli, M. Colombo

Dipartimento Protezione e Valorizzazione Agroalimentare, Università di Bologna, Italy

Corresponding author: Vincenzo Russo. Dipartimento Protezione e Valorizzazione Agroalimentare, Sezione di Allevamenti Zootecnici. Via Fratelli Rosselli 107, 42100 Reggio Emilia, Italy – Tel: +39 0522 290522 – Fax: +39 0522 290523 – Email: vincenzo.russo@unibo.it

RIASSUNTO – Ricerca di geni candidati per il calo di salagione del prosciutto mediante Fluoro Differential Display (FDD). *Con la presente ricerca abbiamo studiato il livello di espressione di geni nel tessuto muscolare di suino con la tecnica fluoro differential display, per identificare geni espressi in modo variabile in funzione del parametro calo di prima salagione. Sono stati identificati dieci geni, dei quali sei a funzione nota e quattro non ancora caratterizzati, che mostravano un'espressione maggiore negli animali con il calo di prima salagione più alto.*

KEY WORDS: pig, salting loss of ham, differential display, candidate genes.

INTRODUCTION – The Differential Display (Liang and Pardee, 1992) is a technique suitable to screen a specific tissue transcriptome without any prior knowledge of the sequences expressed. This method has been used in some experiment aimed to find genes differentially expressed in porcine skeletal muscle (Janzen *et al.*, 2000; Ponsuksili *et al.*, 2000). In the present research we investigated the expression level of genes related to salting loss (SL) of ham by the use of FDD technique. Salting loss is a measure of water-holding capacity under salting condition. This parameter has shown a coefficient of phenotypic correlation of 0.57 (Russo *et al.*, 1991) and a genetic correlation of 0.65 (Buttazzoni *et al.*, 1993) with the seasoning loss of dry-cured ham.

MATERIAL AND METHODS – For this research were slaughtered 277 Large White pigs, 183 females and 94 castrated males. All animals were reared on the same herd, belonging to the National Association of Swine Breeders (ANAS) where they where used for the calculation of the genetic indexes by sib test. The animals were slaughtered on the same abattoir in different days. From each animal, a sample of *semitendinosus* muscle was collected within 1 hour after slaughter and immediately frozen in liquid nitrogen then stored in a -80°C freezer until use. Tacking into account the genetic index for SL used in the Italian selection program, 4 unrelated animals (2 females and 2 castrated males) with the lowest index, and 4 with the highest index were selected. From each sample, total RNA was extracted by Trizol (Life Technologies) and pooled after quantification, using an equal amount of RNA from each animal. The two obtained pools were reverse transcribed with Superscript II (Life Technologies) and amplified with Hieroglyph kit (Beckmann Coulter). The PCRs were performed with 96 primer combinations using 12 oligo dT anchored primers and 8 arbitrary primers. For the fluorescent detection of the bands, Fluoro DD kit (Beckmann Coulter) was used. For both kits the standard protocol was used. The PCR samples were electrophoresed on denaturing 4.5% polyacrilamide gel, using the Genomyx LR DNA sequencer (Beckmann Coulter). After electrophoresis bands were visualized using Genomyx SC apparatus (Beckmann Coulter). Differential bands were excised from the gel and directly amplified using the same primer pairs utilised for the FDD. The amplified bands were cloned with Topo TA cloning kit (Invitrogen) and the plasmids were directly sequenced using universal primers. To identify the genes the sequences were analysed using FASTA (http://www.ebi.ac.uk/fasta33/).

RESULTS AND CONCLUSIONS – The FDD protocol utilized to identify SL-related genes gave fingerprints composed of about 50 well distinct bands in the size range of 200-1500 bp. On the whole about 5000 different cDNAs fragments were separated and analysed. Among these bands, we found 12 strongly differentially–expressed bands, always over expressed on the pool with the highest weight loss, which were designated 1-12. Sequences were obtained for 11 clones. FASTA analysis allowed the identification of such clones (Table 1). Similarity was found to 6 known genes, to 4 gene with an uncharacterized function, and to one L1 interspersed element.

Table1. Results of the homology search performed by FASTA of the 11 identified clones. The most significant match is reported.

Clone	Estimated bp	Name	Species (a)	Matching sequences Accession No.	E value	Identity (%)	bp overlap
1	640	Sarcolipin (*SLN*)	OC	U96091	9.1e-96	81.3	599
3	730	Protein tyrosine phosphatase type IVA, member 2 (*PTP4A*)	HS	BC070181	1.1e-172	94.3	718
4	780	mRNA for Titin protein (*TTN*)	HS	X90569	1.3e-167	91.5	751
5	670	Ubiquitin protein ligase E3A (*UBE3A*)	HS	BC002582	3.5e-112	93.1	680
6	1200	*DSCR1*-like protein mRNA	OA	AY205232	1e-104	81.6	1067
7	1020	Triadin (*TRDN*)	HS	AJ489257	1.6e-76	61.7	543
8	660	cDNA FLJ37781	HS	AK095100	5.8e-35	77.0	548
9	1000	*Sus scrofa* trancribed locus	SS	CJ010134	2.2e-114	87.6	637
10	870	*Sus scrofa* genomic clone (L1 element)	SS	CR863371	3.9e-125	98.4	548
11	700	Sus scrofa transcribed locus	SS	CN159209	2.6e-175	99.7	647
12	690	Hypothetical protein MGC35048	HS	AK093894	2.1e-73	76.5	673

(a) HS, *Homo sapiens*; OC, *Oryctolagus cuniculus*; OA, *Ovis aries*; SS, *Sus scrofa*.

The genes Sarcolipin (*SLN*), Triadin (*TRDN*), Titin (*TTN*) and Down syndrome critical region 1 (*DSCR1*) are mostly expressed in the skeletal and cardiac muscle and could be involved in the determination of meat quality in pigs. Both Sarcolipin and Triadin were isolated in the sarcoplasmic reticulum. Triadin is an integral membrane protein of the junctional sarcoplamic reticulum that binds to calsequestrin and anchors it to the ryanodine receptor 1 (*RYR1*) in skeletal muscle (Kobayashi *et al.*, 2001). The porcine triadin gene has been assigned to chromosome 1 (Dusher *et al.*, 1998). Sarcolipin is a low molecular weight protein that control the activity of the *SERCA1*, which is responsible of muscle relaxation by the reuptake of Ca2+ (Asahi *et al.*, 2004). In pigs *SLN* has been assigned to chromosome 9 (Fontanesi *et al.*, 2001). The giant protein titin is the third most abundant protein of vertebrate striated muscle after myosin and actin. Titin plays a key role in muscle assembly, force transmission in the sarcomere Z-line, and maintenance of resting tension in the sarcomere I-band (Itoh-Satoh *et al.*, 2002). Porcine titin gene has been mapped on chromosome 15 (Bertani *et al.*, 1999). There is some evidence of a relation between the reduced rate of postmortem degradation of structural proteins such as titin and the lower quality and protein functionality of muscle from stress-positive pigs (Boles *et al.*, 1992). Moreover, Melody *et al.* (2004) found that the rate of post-mortem degradation of titin is associated with differences in pork tenderness and WHC. Down syndrome critical region 1 (*DSCR1*) and *DSCR1*-like proteins, are a family of small, structurally related proteins that are preferentially expressed in heart, skeletal muscle and brain (Fuentes *et al.*, 2000). In pigs *DSCR1* has not been characterized yet, but some sequences related to this gene are available on Genbank and classified as Ssc.3189 in Unigene. As the metabolic pathway related to this gene has been suggested to be related to cardiac and skeletal muscle hypertrophy (Musaro *et*

al., 1999; Semsarian *et al.*, 1999), *DSCR1*-like protein could be involved in meat quality determination. No relations with meat quality can be found in literature for the other 2 genes, Ubiquitin protein ligase E3A (*UBE3A*) and Protein-tyrosine phosphatase type 4A (*PTP4A*).
In conclusion, by FDD, we isolated a group of putative candidate genes that can be responsible for SL. Before to consider these genes for association studies, these results needs to be validated by an alternative method of evaluation of gene expression, like Northern hybridization or Real Time PCR.

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