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Short communication

A collection of bovine cDNA probes for gene expression profiling in muscle

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Abstract

Array technology has been increasingly used to monitor global gene expression patterns in various tissues and cell types. However, applications to muscle development and pathology as well as meat production in livestock species have been hampered by the lack of appropriate cDNA collections. To overcome this problem, a directed cDNA library was constructed starting from 23 muscles of meat-producing bovines to derive a collection of 3573 clones. A preliminary sequence characterization of this collection indicated that the most abundant transcripts correspond to genes encoding proteins involved in energy metabolism (COX and NADH dehydrogenase subunits) and belonging to the contractile apparatus (myosin chains and troponin isoforms). From this cDNA library, we selected a set of 435 clones representing 340 unique genes, of which 24 were novel. This collection was subsequently completed with 75 specific cDNA probes for genes of interest already studied in our laboratory. The bovine 'muscle' cDNA repertoire thus designed was spotted onto a nylon membrane (macroarray) in order to test its utility to further investigate the transcriptome of bovine muscles in relation to meat quality traits. It is also anticipated that this type of collection might be useful for the study of chronic myologic diseases in other mammalian species, including humans.

Keywords: Meat; Quality; Bovine; Library; Transcriptomic analysis

1. Introduction

In all mammals, skeletal muscle is composed of an extracellular matrix rich in collagen [1] and of muscle fibers with different functional properties optimized for different tasks [2]. Number and size of muscle fibers determine the final muscle mass [3]. Therefore, many studies have been carried out in humans, pigs, poultry and cattle to understand the basic biological mechanisms which control muscle growth and characteristics [4]. A large number of studies, which include sequence, gene mapping and expression profiling, have been performed to define the pathophysiological cascades involved in human myogenic diseases [5,6]. In addition, there are many potential applications of genomics in meat science and they seem to be very promising in pigs [7], poultry [8] and cattle [9]. Furthermore, systematic studies of gene expression patterns using cDNA arrays provide a powerful tool to understand the molecular basis of cellular and tissue functions by defining expression profiles of tens of thousands genes at a time [10]. Unfortunately, the development of genomic studies has been delayed in meat-producing animals due to the lack of livestock-specific muscle expressed sequence data. This is one of the reasons why the first study on the bovine muscle transcriptome was performed in a heterologous system [11] using a human muscle repertoire [12]. The only bovine cDNA libraries available when we started this work had been constructed by the US Meat Animal Research Center (MARC) with RNA pooled from multiple tissues [13] including two glycolytic muscles (*Longissimus thoracis*

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and *Semitendinosus*). These libraries were used to develop a high-density cDNA microarray for cattle [14]. Concomitantly, a cDNA microarray was constructed from two cattle cDNA libraries derived from one glycolytic muscle (*Longissimus thoracis*) and subcutaneous fat of an Angus steer [15]. Also, a collection of differentially expressed genes in double-muscled vs normal-muscled embryos was constructed and used to produce cDNA arrays [16]. However, except for two of those libraries constructed with RNA extracted from whole bovine embryos [13,16], the available cDNA repertoires were built without any oxidative muscle, and none of them had been used for monitoring gene expression profiles in muscles.

Therefore, we decided to construct a bovine-muscle specific cDNA library and to derive from it a specific set of muscle gene probes relevant to muscle growth, muscle pathologies and animal meat quality studies. To achieve this goal, muscle tissues from various French bovine breeds producing meat of different qualities under different rearing systems were used. To maximize the potential identification of transcripts expressed at different stages of growth, muscles were sampled from 23 different sites, with different physiological, age, nutritional and genetic status. In addition, to ensure that all essential genes would be represented in our set, specific cDNA probes of known genes involved in muscle characteristics related to meat quality were selected based on our previous experience [17]. These two complementary approaches (i.e. a panel of specific known genes plus construction of a muscle cDNA library) were combined to obtain a broader and informative cDNA panel.

Such a set of cDNAs is a prerequisite for gene expression profiling studies in bovine muscle to ultimately correlate gene expression patterns with meat characteristics. More generally, it may have applications in other fields. For instance, the metabolic profile of red versus white muscle may be protective against the development and progression of chronic diseases. Individuals with a higher percentage of red fibers are less likely to have chronic metabolic syndromes, such as insulin resistance [18]. In addition, understanding the consequences of muscle growth induced by physical activity is useful to adapt the training and nutrition of athletes. It was thus anticipated that the data resulting from a global gene expression profile in bovine muscle could be useful to characterize different muscle types at various growth stages in order to improve the quality of meat and to forecast chronic myologic diseases of humans. The assembly and validation of the first bovine muscle array designed to identify and profile gene expression in bovine muscle is reported here.

2. Materials and methods

2.1. Preparation of RNA muscle samples

Twenty-three different muscles were used to construct the cDNA library (Table 1). Muscle samples from freshly

Table 1
Muscles collected to make the RNA pool from which the bovine muscle cDNA library was constructed

Metabolic type	Tissue	Age	Breed
Heart (the most oxidative muscle)	H 1	110 dpc	Charolais×Salers
	H 2	110 dpc	Double-muscled INRA 95
	H 3	260 dpc	Charolais×Salers
	H 4	260 dpc	Double-muscled INRA 95
	H 5	15 days of age	Montbéliard
	H 6	1 month of age	Holstein×Friesan
	H 7	calves	Holstein
	H 8	calves (pool)	Holstein
Oxidative muscles	Ma 1	110 dpc	Charolais×Salers
	Ma 2	260 dpc	Charolais×Salers
	Ma 3	15 days of age	Montbéliard
	Ma 4	young bull (15 months of age)	Charolais
Glycolytic muscles	CT 1	110 dpc	Charolais; Double-muscled INRA 95
	CT 2	180 dpc	Charolais; Double-muscled INRA 95
	CT 3	210 dpc	Charolais; Double-muscled INRA 95
	CT 4	260 dpc	Charolais; Double-muscled INRA 95
	LT 1	15 days of age	Montbéliard
	LT 2	1 month of age	Holstein×Friesan
	LT 3	young bull (15 months of age)	Charolais
	ST 1	110 dpc	Charolais×Salers; Double-muscled INRA 95
	ST 2	260 dpc	Charolais×Salers; Double-muscled INRA 95
	ST 3	young bull (15 months of age)	Charolais
	ST 4	young bull (15 months of age)	Charolais

dpc, days post conception; H, heart; Ma, Masseter; CT, *Cutaneus Trunci*; LT, *Longissimus thoracis*; ST, *Semitendinosus*.