Histochemical profiles and quantitative analysis of muscle fiber types in different positions of cultured Pacific bluefin tuna (*Thunnus orientalis*)

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Tuna meat is a delicacy and widely eaten raw as *shashimi* in Japan. Muscle fibers are the main structural unit and the main contributor of the chemical, physical, nutritional and textural properties of muscle. Muscle fiber numbers size and their distributions are often referred as an important determinant of fish flesh quality. These traits are of interest to potential breeding programs since the final number of fast muscle fibers for other aquaculture species, such as Atlantic salmon, is known to vary between families and populations, has a moderate heritability and correlates with growth rate. It is well known that body growth in fish continues throughout life and that both the number of muscle fibers and their diameters (size) increase steadily. In fish, muscle growth occurs by from small type I to large type II or by hyperplasia or by hypertrophy of muscle fibers. Muscle fiber density was positively correlated with an increase in the firmness of the flesh and high fiber density were associated with a reduction in the incidence of fillet gaping. The fish flesh comprises three different muscle tissues: red superficial, deep white muscle and intermediate pink muscle, which differs in their metabolic and contractile properties. Also, the axial musculature of teleosts has been classified as white and red with reference to muscle color and further subdivision with more fiber types has been reported.

The edible muscular tissue of tuna is frequently marketed as three distinct cuts (*chu-toro, akami, o-toro*) identifiable by location, lipid content and color. Chemical composition of cultured tuna muscle tissues indicates that there are significant differences between these cuts. But there is no study on muscle fiber type composition of cultured tuna on different commercial cuts. Therefore, this study was taken consideration to investigate the muscle fiber distribution in trunk muscle and cellular components characteristics of muscle fibers of cultured Pacific bluefin tuna.

Materials and methods

Fish

Cultured Pacific bluefin tuna at their live weight at 10.7 ±0.9 and 15.9 ±1.0kg was used in this study.

Muscle sampling

Muscle sampling was done by dissecting the trunk of tuna flesh in the cephalal part and make slices (Figure 1). Then the slices were divided into different cuts as shown in the figure 1 and each cut was divided into 5 blocks and numbering the blocks 1, 2, 3, 4 and 5 from the

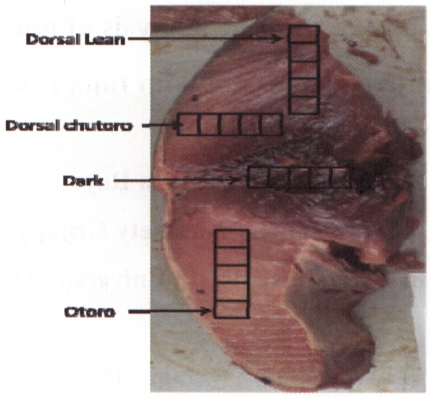

Figure 1. Sampling positions (dorsal lean, dorsal *chu-toro*, dark and *o-toro*) of cultured tuna muscle.

outer side to the median line. One muscle sample block from each number were frozen with dry ice acetone mixture and stored at 50°C until used for histological preparation. Another muscle sample block from each number was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer for some days in refrigerator at 4 °C for Transmission Electron Microscopy.

Histochemical methods

Serial frozen sections (8μm thick) were stained by histochemical reactions for myosin adenosine triphosphatase (ATPase) activities after acid (pH4.3) or alkaline (pH 9.4, 10.3, 10.5) pre-incubation and reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-DH) activity .

Transmission Electron Microscopy (TEM)

Cellular components of muscle fiber will be analyzed from the photographs by Transmission Electron Microscopy observation. This was not yet completed.

Results

Histochemical fiber types

Myofibers with a positive reaction for alkaline-ATPase activity and a negative for acid-ATPase were designated as Type IIB. Type II fibers were divided into Type IIC (Intermediate) showing the medium NADH-DH activity and Type IIA with the highest activity. In Type IIA fibers the granules were scarcer in the central region and gathered around the peripheral. The very small granules distributed throughout Type IIC fiber indicated very weak NADH-DH activity.

Conclusion

The data of this study are not yet analyzed. In this study, myofiber types and myofiber cross sectional area will be measured and analyzed. The structure of cellular components will be studied by Transmission Electron Microscopy to investigate the whole trunk muscle characteristics of cultured tuna. We could not be able to conclude anything of the results of the present study in this report

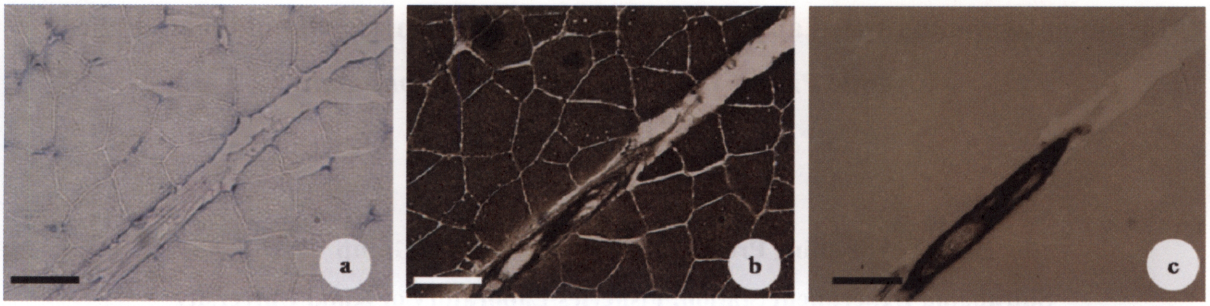

Fig. 2. Cross section of the dorsal *chu-toro* muscle of cultured tuna (a) NADH-DH activity, (b) m-ATPase activity after alkaline pre-incubation at pH 9.4, 10.3, 10.5, (c) m-ATPase activity after acid pre-incubation at pH 4.3. All muscle fibers are categorized as type IIB. Bar =100μm

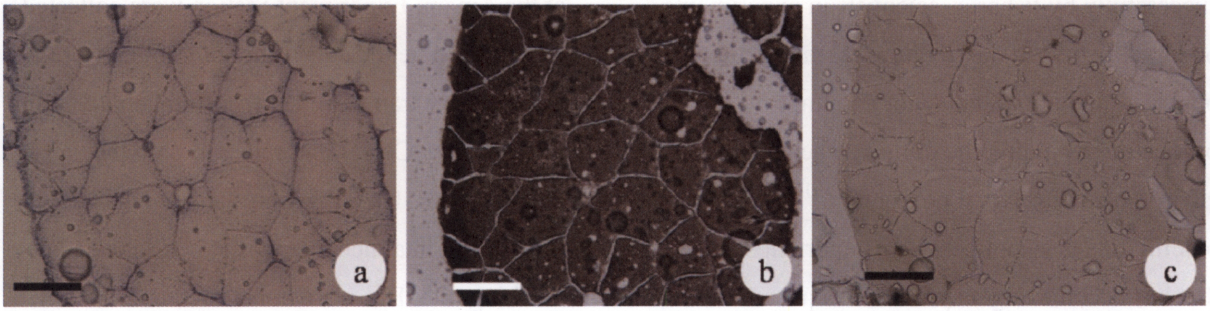

Fig. 3. Cross section of the *o-toro* muscle of cultured tuna (a) NADH-DH activity, (b) m-ATPase activity after alkaline pre-incubation at pH 9.4, 10.3, 10.5, (c) m-ATPase activity after acid pre-incubation at pH 4.3. All muscle fibers are categorized as type IIB. Bar =100μm

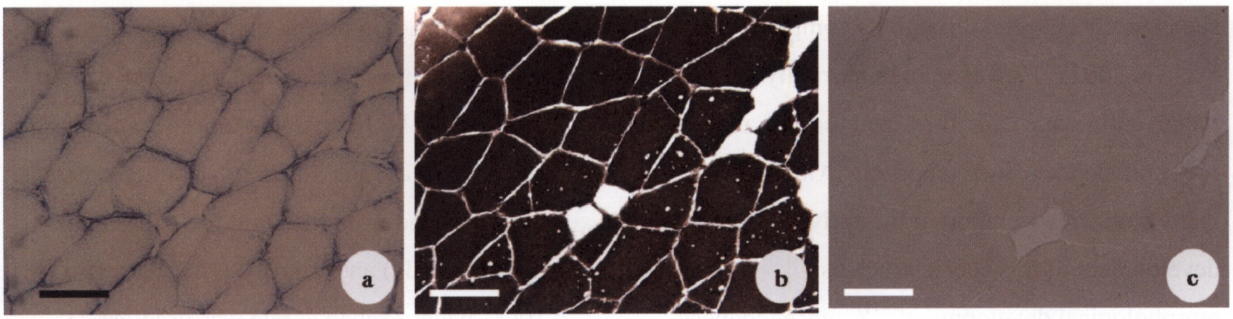

Fig. 4. Cross section of the dorsal lean (dorsal *akami*) muscle of cultured tuna (a) NADH-DH activity, (b) m-ATPase activity after alkaline pre-incubation at pH 9.4, 10.3, 10.5, (c) m-ATPase activity after acid pre-incubation at pH 4.3. All muscle fibers are categorized as type IIB. Bar =100μm

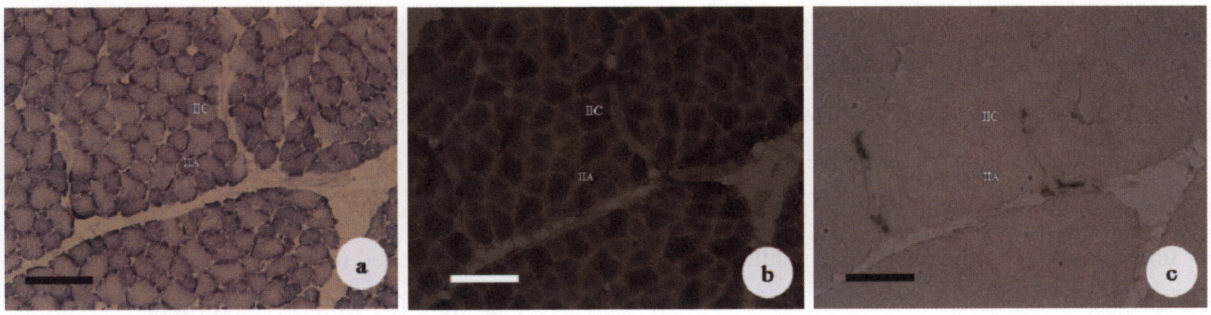

Fig. 5. Cross section of the dark muscle of cultured tuna (a) NADH-DH activity, (b) m-ATPase activity after alkaline pre-incubation at pH 9.4, 10.3, 10.5, (c) m-ATPase activity after acid pre-incubation at pH 4.3. myofibers are categorized as type IIA with stronger NADH-DH activity and medium alkaline m-ATPase activity and negative acididc m-ATPase activity and as type IIC with medium NADH-DH activity and stronger alkaline m-ATPase activity and negative acididc m-ATPase activity. Bar =100μm.