DNA Extraction from Leather

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Abstract

The identification of animal species on leather is mainly made with microscopic observation on their surface and cross section. Microscopic observation, including scanning electron microscope and light microscope, provides great detail of grain pattern and anatomical structure. However, some kinds of leather, such as split leather, corrected leather, and very thin leather are difficult to identify its animal species.

Analysis of DNA is a relatively new and developing field of study in biotechnology, but this method has proven to be an effective tool in some fields. Since DNA analysis is the most popular methods for species identification of animal specimens at present, it is expected as an effective method to identify animal species on leather. However, there are few studies on the DNA analysis of leather.

Therefore the present study was focused on the solubilization of the leather by the microbial cleavage and the following extraction of DNA. Commercial seven bovine leather samples were conducted to the microbial degradation step and the following DNA analysis. The results obtained in this study demonstrated that DNA could be extracted effectively by microbial cleavage.

Keywords: DNA extraction, identification, microbial degradation

1. Introduction

Leather is made from the hide or skin of animals such as bovine, goat, sheep, pig, horse, crocodile, lizards, and snake. There are so many different types of leather finishing used in leather, therefore some leather is almost impossible to identify its animal species only by appearance. As specified by ISO 17131:2012 (IULTCS/IUP 56), identification of leather is usually conducted by microscopy such as light microscope or scanning electron microscope. This method provides great detail of surface and cross section of leather, and the identification is based on the differences of the grain pattern, the distribution of the hairs or wool follicles, and the anatomical structure varies among animal species. However there are some difference between animals within a species and the part of sampling. In addition, almost all of leather is treated with various types of finishing and leather is generally split into layers to various thicknesses. Therefore, some kinds of leather are

difficult to identify its animal species. As shown in the ISO 17131, the method is not applicable for identifying specific leathers (e.g. sheep leather). That is, it is difficult to distinguish the leather species of similar livestock by means of its structure.

On the other hand, DNA identification is used as an already established method in the field of meat, fish, and other food. However, there have been few studies on the DNA analysis of leather identification (Yoshioka 2006; Vuissoz et al. 2007; Hans 2009). The reasons for this are as follows.

There are very few DNA containing in dermis.
DNA is partially destroyed by some chemicals during leather processing steps.
As DNA is tightly linked with collagen fibers, it is difficult to extract from leather.

The first and the second reasons are invariable factor determined by their material and leather processing. It remains possible that DNA may be extracted from leather by solving a technical problem with the third factor, if it is still present in leather. In other words, if we are able to solubilize leather effectively, it has the potential to extract DNA from leather. Although the methods for extracting DNA by using collagenase (Yoshioka 2006) and enzyme (Hans 2009) have been previously reported, it is insufficient results to determine its animal species. The purpose of this paper is to investigate the possibility of DNA identification with extraction of DNA from leather using collagenase-producing microorganisms.

2. Material and Methods

Material

Various finished seven bovine leather samples were obtained from commercial sources. For leather degradation, *Vibrio hollisae* 1706B was used as a producer of collagenolytic enzymes, and was incubated in gelatin plate for 6 hours before use. The resultant culture solution was used for leather degradation.

DNA extraction

Chrome is known to act as an inhibitor of decomposition by inactivating enzymes; therefore leather samples were treated to remove the chrome. Citric acid was used to minimize the damage of the DNA for DNA amplification. 200 mg of the leather pieces in a micro tube with 1 ml of 0.5% citric acid were stirred at 50 °C for 24 hours. After the neutralization with phosphate buffer (pH 7.0) solution after the growth of microorganisms was added to tube, then the mixture was stirred using a tube rotator. The tubes were then centrifuged at 15,000 rpm for 10 min, the supernatant was removed and the precipitation was recovered and homogenized with homogenizer. DNA was isolated from them using the DNA extraction kit (QIAamp DNA Stool Mini Kit, QIAGEN). The isolated DNA was further concentrated by ethanol precipitation. Treatment with collagenase and proteinase K were used as a control.

DNA analysis

The presence of DNA was assessed in all samples using polymerase chain reaction (PCR) amplification. Amplified site was the cytochrome b gene region of mitochondrial DNA. The nucleotide sequences of the primers were the primer of the meat identification as described by Matsunaga et al. (Matsunaga 1999). PCR products were analyzed by electrophoresis on a 10% acrylamide gel stained with SYBR Gold and subsequently visualized on an ultraviolet irradiation. Stained gels were photographed with a digital camera.

3. Results and Discussion

In this study, DNA extraction was conducted to various finished bovine leather samples. Pretreatment of the citric acid, the following microbial degradation and homogenization significantly dissolved the leather samples. As shown in Figure 1, DNA could be successfully amplified at 289 bp from six among the seven samples at specific amplification region on the basis of the primer of meat identification. However, the results of amplification of collagenase digestion showed only one out of the seven samples (Figure 2). These results suggested that the microorganisms produced various enzymes and digested leather samples in multiple steps. Therefore, it is considered that the DNA could be extracted more effectively by the microorganisms.

In this microbial degradation, DNA could be efficiently extracted without the need to grind or cut into small pieces and to remove the coating of the leather surface. On the other hand, there is a problem that it is hard to manage the microorganism. In the future, it is necessary to develop technology to facilitate the management and handling of microorganisms in order to popularize this method. We are now under investigation whether it would be possible to available to another animal species including horse, sheep, goat, and reptile. Further experiments will be carried out in the future.

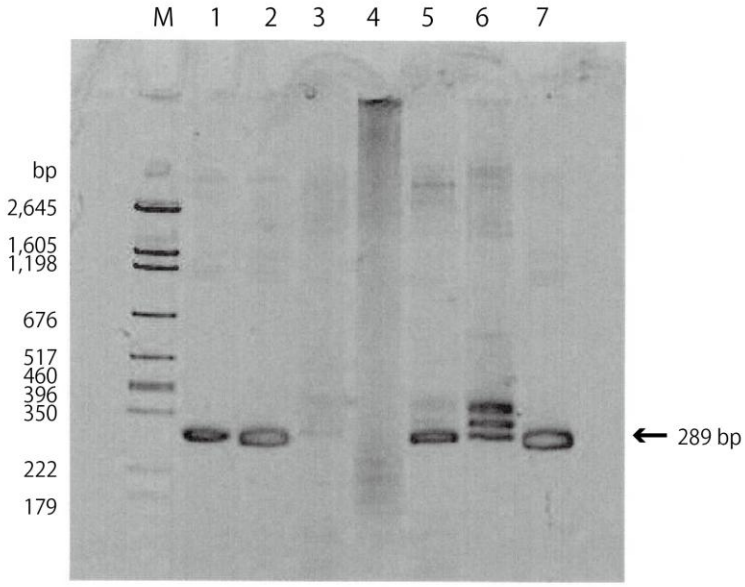

Figure 1. Results of agarose gel electrophoresis of PCR products by microorganisms degradation
M: DNA marker, 1 - 7: bovine hide

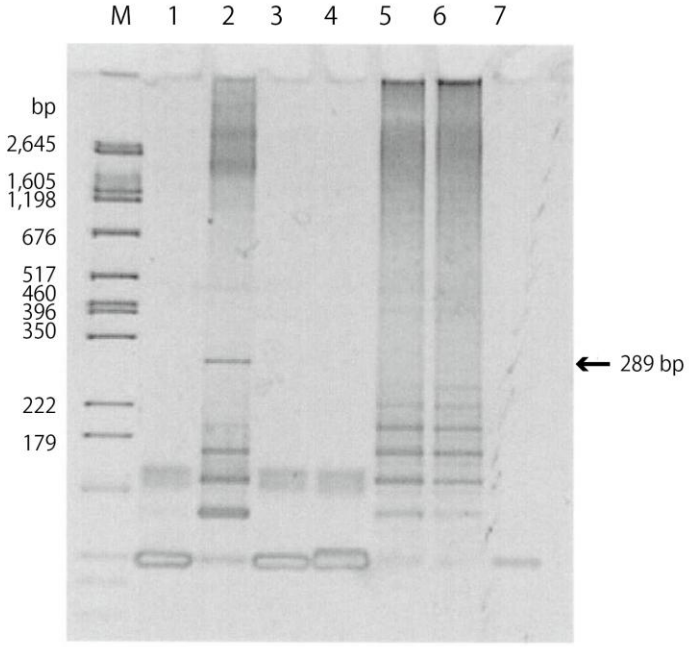

Figure 2. Results of agarose gel electrophoresis of PCR products by collagenase digestion
M: DNA marker, 1 - 7: bovine hide

4. Conclusion

We investigated the DNA extraction from various finished bovine leather samples. Pretreatment of the citric acid, the following cleavage with collagenase-producing microorganisms and homogenization significantly

dissolved the leather samples. From the results of PCR amplification, DNA could be extracted effectively by microbial cleavage. We concluded that the microorganisms' cleavage is useful digestion method to extract DNA effectively. To establish DNA identification of leather, further research is required.

5. Reference

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