DNA ANALYSIS, A NEW METHOD TO IDENTIFY ALL LEATHER ORIGINS?

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Abstract:

Using DNA to trace people who are suspected of committing a crime is one of the biggest advances in tackling crime since fingerprinting. When DNA profiling is used wisely it can bring major benefits to society by helping to convict serious criminals including murderers.

For leather, one current way to identify species consists in analysing the distribution of the hairs or wool follicles. With full grain leather, it is possible to identify different species.
But the market uses lots of corrected grain leathers and leather splits. It is more difficult to identify which species they come from. In this case, DNA analysis could help to find the origin of the leather.

DNA analysis on raw hides and skins do not present any technical problems. When the hides or skins are transformed into leather, the DNA is partly destroyed during the different processing steps.

First results have shown that the tanning process generates inhibiting factors that destroy DNA or act as masking agent of DNA. Therefore, the aim of the project is to develop a method which certifies the origin of the species on finished leather

Key words: Leather, identification, DNA, PCR.

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1. Introduction:

DNA analysis can be used to compare animal species. DNA is the heredity molecule. It contains nucleotides sequences, in which all the information of a living organism is coded.
Each species can be determined regarding the analyse of one or several sequences inside DNA. Research programs on the topic of sequencing lead to a continuous increase of the number of registered sequences in data bases.

This project consists in evaluating the possibilities offered by DNA (Deoxyribonucleic acid) analysis, for animal species identification on hides, skins and leathers.
On raw hide, DNA quality is sufficient for subsequent amplification and analysis.
Once the leather is finished, DNA analysis becomes rather complex,

- because the chemicals are inhibiting the analyze and do not able the amplification,
- or DNA is too damaged by tannery operations,
- or DNA is no more present in a minimal quantity to be analyzed.

The aim of this project has been to developed new methods to solve the problem of inhibition.

2. Experiments:

Genetic inheritance enables species identification. Two different DNA exist in each cell.
The nuclear DNA (from the nucleus) and the mitochondrial one. This research program focuses on mitochondrial DNA, where the amount of DNA is larger than in the nucleus.

The methods to analyze DNA consists in 4 steps :

Leather preparation

From one piece of leather (without finishing on the top), several washings are achieved before grinding.

Lysis of the obtained powder

One gram of leather is mixed with a lysis buffer solution and with an enzyme. After 72 hours rotation, the tube is purified.

DNA extraction

In order to purify the solution from inhibitors, several washings are run with special filters in silica which will capture DNA.

Two different ways to analyze the extracted DNA:

- Either the nature of the product is unknown and it is necessary to proceed to a DNA sequencing. It will consist in amplifying DNA and in comparing the obtained sequences to a bank of sequences where all of the worldwide-known sequences are registered.

- Or the nature of the product is known, it is then possible to use Polymerase Chain Reaction (PCR).
  The PCR is a replication of DNA fragment in order to obtain large amount of target sequence. Instead of being a linear amplification, it is an exponential one.
  Some Real-time PCR systems use fluorescent probes that bound the specific target (related to the known species). This system enables to check easily whether the sample analyzed contains the searched DNA.

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3. Results

DNA can be analyzed when leather is tanned.
To develop the adequate method on semi-finished leather and finished leather, extraction and amplification are the critical steps of the procedure.
The method developed enables to work with 10 mg of dry leather and runs with 3 analyzes simultaneously:
- a negative DNA witness of extraction and amplification in order to control the absence of DNA in the chemical used
- a positive DNA witness for the researched species in order to check to validity of the reactive used
- an inhibitor witness to control the lack or show the presence of inhibitors in the extracted DNA.

The method has been adapted in order to obtain the largest amount of DNA. A minimum of 200 mitochondrial copies is required for the analyse.

Three dilution factors have been studied (10, 50,100). It shows that if some extract contains inhibitors, (even after purification), their dilution can decrease inhibition and able to analyze the DNA extract correctly.

This method has been conducted mainly on one kind of bovine hide.
The reproducibility of the method must be still improved to certify any kind of bovine leather.

4. Conclusion

Identification of bovine species on finished leather using DNA analysis has been successful using Real-Time PCR and sequencing.
Nevertheless, in special cases, when DNA is too destructed, it is not possible to have a good reproducibility. For this reason, specific methods that are dedicated to low DNA content matrixes are being developed.

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