INFLUENCE OF MEDIAEVAL CLOTHES COLOUR PIGMENTS ON DNA EXTRACTION AND AMPLIFICATION

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INTRODUCTION

Different factors affect DNA preservation and recovery such as depth of burial, soil pH, presence of humic acids or pigments of ancient clothes.
We examined bone fragments (vertebra, talus and heel bones) from a mediaeval skeleton burial (810-900 AD) excavated from a burial vault. This skeleton belonged to a duke dressed with mediaeval clothes stained with kermes vermilio binded to the wool by elagic acid and alun [$Al_2(SO_4)_3$]. The vertebra was slightly brown coloured and we showed in a previous (Crubézy, 1995) study that cytochrome b mtDNA had been successfully amplified.
The talus and the heel bone showed red-brown color due either to the kermes vermilio or to the putrefaction processes and the extracts were also brown coloured from the previous contaminants. These extracts could not be amplified.
To investigate the inhibition power of these four contaminants (alun, kermes vermilio, kermes coloured wool and elagic acid), modern DNA was extracted with those pigments.

MATERIALS AND METHODS

DNA extraction : modern genomic DNA (20 μg) was isolated and purified from 1 ml of human blood sample by the organic extraction protocol (Maniatis *et al.* 1982) in absence or presence of 10 mg of different types of coloured clothes contaminant (kermes, kermes coloured wool, alun, elagic acid).

Polymerase Chain Reaction : PCR amplification was performed in 50-μl volume of 20 mM Tris-buffer (pH 8,4) containing 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 0.05 % Tween, 10 μg of bovine serum albumin, 5 pmole of each primer, 10μl of the extracted DNA and 2.5 units of Taq DNA Polymerase (Gibco BRL). Primers used for amplification and sequencing were L14841 and H15149 (Kocher *et al.*, 1989). Each cycle of the polymerase chain reaction, carried out in a Perkin Elmer-Cetus Thermal Cycler, consisted of denaturation for 1 mn at 94°C, hybridization for 1 mn at 50°C, and extension for 2-5 mn at 72°C. This cycle was repeated 35 times.
The PCR products were separated on 3 % NuSieve agarose gels and stained with ethidium bromide.

RESULTS

The DNA extraction was not affected by the component added except when carried out in the presence of 10 mg of alun, which interfered with the DNA recovering by the formation of a precipitate.
Following agarose electrophoresis and ethidium bromide staining, PCR products of expected size were detected with the DNA extracted without contaminant and with kermes coloured wool and kermes. Faintly PCR product was observed for the DNA extracted in the presence of alun. A band of smaller size than the region of the cytochrome b gene flanking by the two primers used was also observed and was probably due to a DNA degradation. No amplification product occurred with the DNA extracted in the presence of 10 mg of elagic acid.
In order to test the influence of the elagic acid concentration and to determine a possible inhibitory threshold value, DNA extraction was carried out with seven different concentrations of elagic acid (0,5; 1; 3; 6; 8; 10; 16 mg) in the experimental conditions primary used.
As shown in the figure, fewer products appeared with increasing elagic acid concentration in the extraction solution. No visible bands were detected beyond 10 mg of elagic acid.

DISCUSSION

After death, the conservation of DNA depends mostly on either environmental factors or artificial treatment of the body. Humidity combined with anaerobic conditions and a high concentration of humic acids may lead to complete conservation of the body (Hauswirth et al., 1994).
If the soft tissues are decomposed, the organic structures in bones may persist even under normal burial conditions. This observation can be explained by the situation in little caves of the osteocytes or osteoblastes which are surrounded by protective hard tissues protecting the DNA against physical and biochemical aggressions.
In previous studies, it was shown that this mediaeval crypt burial belonged to a count of Toulouse (France). In fact, one of the skeletons was dressed with rich mediaeval clothes and the lord was wearing red socks. We showed that the extracted DNA from the vertebra was suitable for PCR and a part of the cytochrome b gene could be amplified. On the opposite, the DNA yielded from talus and heel bones could not be amplified. Macroscopically these bones showed a brown-red colour coming either from the degradation processus or from the coloured clothes contaminants. In the present study, we tried to highlight the effects of these tannins on the DNA molecule by adding 10 mg of the four contaminants (kermes, kermes coloured wool, alun and elagic acid) to the extracted modern genomic DNA.
Kermes seems to have no visible effects, neither on the DNA extraction, neither on the amplification reaction. In contrast, our results suggest that alun alters the DNA extraction and the efficiency of the amplification reaction whereas elagic acid might have inhibitory effects on the amplification reaction.

CONCLUSION

This study allowed us to conclude that the lack of amplification results on talus and heel bones might be attributed to the coloured mediaeval clothes tannins which contamine the bone DNA and inhibit the amplification reaction.

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Figure legend :

Conventional 3 % NuSieve agarose-gel electrophoresis of the amplified products after staining with ethidium bromide. Part of the cytochrome b gene was amplified from human genomic DNA extracted without any contaminant (lane 1) or with 10 mg of vermilio kermes (lane 2), 10 mg of kermes coloured wool (lane 3), 10 mg of alun (lane 4), 0.5 mg of elagic acid (lane 5), 1 mg of elagic acid (lane 6), 3 mg of elagic acid (lane 7), 6 mg of elagic acid (lane 8), 8 mg of elagic acid (lane 9), 10 mg of elagic acid (lane 10), 16 mg of elagic acid (lane 11). Lane 12 denote a DNA mass ladder marker (Gibco BRL).