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SHORT COMMUNICATION

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Influence of soil storage and exposure period on DNA recovery from teeth

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Abstract A study was performed to determine the influence of garden soil on the deoxyribonucleic acid (DNA) recovery from teeth depending on the duration of storage. In the first series 24 teeth supplied by dentists were exposed to garden soil storage for a maximum of 18 weeks. Selected samples were excavated for DNA extraction at time intervals of 6,12 and 18 weeks. For the second series 20 teeth were stored for one year in garden soil. Following phenol/chloroform extraction with decalcification (first series) and without decalcification prior to extraction (second series) DNA was quantified, amplified using the polymerase chain reaction (PCR) for the tandem repeat loci D1S80, tyrosine hydroxylase, intron 1 (TH01) and Von Willebrand factor, intron A (VWA) (first series), human alpha fibrinogen (FGA) (second series) and sequenced in the hypervariable regions 1 and 2 (HV1, HV2) of the mitochondrial DNA (second series). The DNA concentration of the extracts after the first 6 weeks in soil was reduced by more than 90%. Amplification and direct sequencing of HV1 and HV2 of the mitochondrial genome was the most successful DNA technique.

Key words DNA extraction · Short tandem repeat (STR) systems · Mitochondrial DNA · Teeth

Introduction

In several spectacular forensic cases, DNA extracted from bone has been used to identify skeletal remains (Jeffreys et al. 1992; Holland et al. 1993; Gill et al. 1994). In the postmortem period DNA has a limited life depending among others on environmental conditions, and its protection against destructive factors (Bär et al. 1988; Woodward et al. 1994a). Therefore DNA typing from old skeletal material is not always successful and usually only the positive cases are reported. DNA in teeth is more protected than in bones (Woodward et al. 1994b). The dental hard tissues surrounding the pulp chamber seem to physically protect this tissue. Experimental DNA recovery from teeth reflects the rapid development of related technologies (Schwartz et al. 1991; Pötsch et al. 1992; Alvarez Garcia et al. 1996). The aim of this study was to investigate the influence of soil storage on DNA recovery from isolated teeth. Furthermore we wanted to explore the value of the time-consuming decalcification step prior to DNA extraction.

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Materials and methods

First series

A total of 24 teeth from 12 patients (two incisors from each of four patients and two molars from each of eight patients) obtained from dentists after extraction were stored about 30 cm deep in garden soil for a maximum of 18 weeks during the winter period at temperatures between –10° C and +15° C. Prior to burying one tooth from each person was cut into two halves, of which one was stored at –20° C and the other was buried together with the remaining teeth. After 6 weeks the halved teeth were excavated and after 12 or 18 weeks the remaining teeth were recovered. The tooth fragments were washed, ground to a powder and weighed. DNA was extracted according to Hochmeister et al. (1991) with a 4-day decalcification step prior to extraction, quantified using the slot-blot method (Waye et al. 1989) and amplified for the locus D1S80 (Kasai et al. 1990) and the STR systems TH01 (Edwards et al. 1991) and VWA (Kimpton et al. 1992). Of the DNA extract 10 µl was added to the 25 µl reaction mix containing AmpliTaq DNA polymerase (Perkin-Elmer). PCR products were separated in 8% horizontal polyacrylamide gels and stained with silver (Budowle et al. 1991).

Second series

A total of 20 teeth obtained from dentists after extraction from patients were stored 50 cm deep in garden soil for one year. All types of teeth and all kinds of tooth destruction were present and in some cases only parts of the roots were left. Bloodstains from the patients were collected for comparison in all cases during tooth extraction and stored until use. After cleaning the surface, crushing

and weighing the samples, DNA was extracted using a conventional phenol extraction method without a prior decalcification step (Smith et al. 1993), quantified using the slot-blot method (Waye et al. 1989), amplified in the FGA system (Barber et al. 1996) and sequenced in HV1 and HV2 of mtDNA following the instructions of the Armed Forces DNA Identification Laboratory, Rockville MD, USA (AFDIL). Of the DNA extract 2.5 µl was used for the 25 µl PCR reaction mix.

DNA was extracted from the bloodstains using the Chelex method (Walsh et al. 1991), amplified and sequenced in the same manner as the tooth extracts.

In both series the patients were informed about the planned investigations and consent was obtained from all patients before starting the experiments.

Results and discussion

First series

The DNA concentrations obtained from the tooth halves showed a rapid decrease over the first period (Table 1). Because of the longitutinal section the pulp was opened and the tissue exposed to the environment. Therefore the DNA of the pulp cells was degraded faster than in teeth which were not divided. The amplification of D1S80 was successful in the frozen samples, but gave no results after 6 weeks in soil. This suggests that fragment lengths more than 500 bp cannot be amplified successfully in old skeletal material or decomposed bodies. The STR systems TH01 and VWA gave better results. After 18 weeks, amplification was successful in 4 (VWA) or 2 (TH01) out of 12 cases and there existed a correlation to the duration of soil storage (after 6 weeks 8 were positive in VWA, 7 in TH01 and after 12 weeks 6 were positive in VWA and 5 in TH01).

Second series

After one year three extracts (out of 20 teeth) did not contain sufficient human nuclear DNA to be detected using the slot-blot technique (Table 2) and of these two also failed to give positive STR products. The DNA concentration in the extracts did not depend on the weight of the tooth powder used for DNA extraction. The amplification of mtDNA in HV1 and HV2 was successful in all 20 samples and the sequences obtained were identical to the controls (data not shown here). Therefore, mitochondrial DNA analysis seems to be the most efficient method for the identification of old skeletal remains.

The best results for DNA extraction were obtained in the second series although in contrast to the first study the samples were stored for a much longer period in soil and in many cases the crown was destroyed by caries and the pulp chamber opened. We attribute this to the omittance of the decalcification step prior to DNA extraction which makes the extraction much easier and results in a greater yield of DNA, or in a decreased loss of DNA during extraction. Our results correspond to Fisher et al. (1993), who investigated bone samples and found that decalcification is not a necessary step in the extraction process and that the yield of DNA is 2 times as much when decalcification is omitted. This method was also successful for DNA extraction from old bone and tooth material at the AFDIL (personal communication). But there also existed other differences between both series which can have influenced the different efficiency rates. In the first series we used the maximum of DNA extract while in the second series only 2.5 µl of the extract was used. Therefore, higher concentrations of enzyme (Ampli Taq DNA polymerase) inhibitors in the extracts of the first series can be another reason for the differences observed.

The results of our study show that isolated teeth, even with a high degree of destruction, are a lucrative source of

Table 1 DNA concentrations extracted from teeth after different intervals of storage in garden soil

Person	Tooth	Native teeth (ng/µl)	6 weeks in soil (ng/µl)	18 weeks in soil (ng/µl)
1	I	8	0.025	0.002
2	I	18	0.050	0.010
3	I	12	0.050	0.004
4	I	20	0.025	0.003
5	M	30	0.100	0.005
6	M	18	0.750	0.400
7	M	10	0.000	0.005
8	M	10	5.000	1.000
9	M	1	0.000	0.005
10	M	30	0.075	0.150
11	M	9	0.100	0.020
12	M	9	0.750	0.004

I - Incisors
M - Molars

Table 2 DNA concentration from tooth extracts and PCR efficiency in the FGA system after a 12-month storage interaval in garden soil (second series). Positive FGA results are classified by the intensity of the bands in the gel

Person	DNA concentration (ng/µl)	FGA
1	0.031	strong
2	0.091	strong
3	0.063	strong
4	0.125	strong
5	0.016	strong
6	0.016	strong
7	0.016	none
8	0.063	strong
9	0.000	none
10	0.000	none
11	0.016	strong
12	0.016	strong
13	0.250	strong
14	0.188	strong
15	0.125	strong
16	1.000	strong
17	0.125	strong
18	0.188	strong
19	0.000	weak
20	0.063	strong

DNA and that the DNA extraction from tooth tissues is more effective when the prior decalcification step is omitted.

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