Evaluation of DNA Extraction and Amplification Protocols to Investigate Bacterial Communities on Cultural Heritage

[1]A. Meloscia, M. Orsini, [1]G. Lustrato, and [1]G. Ranalli
[1]DISTAAM, Università degli Studi del Molise via De Sanctis, 86100 Campobasso (I)

Correspondence to: Giancarlo Ranalli, DISTAAM, Università degli Studi del Molise via De Sanctis, 86100 Campobasso, Italy.
Tel. +39-0874-404604 (fax –652).
E-mail: ranalli@unimol.it

Introduction

The deterioration process of inorganic materials induced by microorganisms, including glass, marble, concrete, sandstone, tufa, and metals, can cause various alterations such as crust formation on surfaces, change of hue, discoloration, and loss of materials, leading to structural damage (Figure 1). These severe problems are due to the excretion of aggressive metabolic products such as organic or inorganic acids during microbial growth. With this excretion material components can be used as substrates for microbial metabolism [1,2].

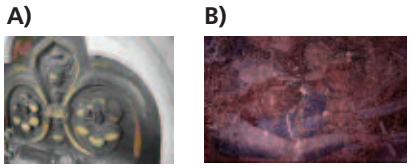

Figure 1. *Alteration of art objects induced by microorganisms. Left: archeological site A; Right: archeological site B.*

For the proper conservation and restoration of our cultural heritage, it is necessary to identify the complete microbial diversity present on art objects. Microbial investigations conducted in the past were based on traditional cultivation studies. The main limitation of this method is the bias toward identifying culturable microorganisms: these techniques recover less than 1% of the total microorganisms present in environmental samples. This approach could lead to serious errors of interpretation, i.e. reflecting the microbial diversity present in samples.

The application of culture-independent techniques on art objects, based on molecular biological methods (especially on the PCR amplification of 16S rRNA genes), avoids the problems of conventional cultivation methods [3]. Direct extraction of DNA introduces less bias than methods in which cells are separated from sample matrix before DNA extraction. DNA from environmental samples often contains PCR inhibitors such as salts, pigments, exopolysaccharides, humic acids, and other unknown substances that are co-extracted with nucleic acids. The quality and purity of the extracted nucleic acid pool is vital for the successful PCR amplification of target genomic DNA/RNA. Different procedures have been tested to extract nucleic acids directly from cultural heritage samples.

Materials and Methods

Samples were collected in two differents sites (A-B, Figure 1), removing alteration by sterile blade. Fragments were ground into a fine powder by mortar and pestel, and 50 milligrams were used for DNA isolation. For each protocol, initial steps of lysis were repeated twice to maximize yield. Quantity and quality of extracted DNA was checked by spectrophotometric assay. DNA concentration was normalized among samples.

Plant GenElute™ – Samples were suspended in 175 µl of lysis solution A and 5 µl of lysis solution B, and then incubated for 20 minutes at 65 °C in a thermomixer (Eppendorf) set at 1,200 rpm. Afterward, centrifugation lysis solution was gently collected in a new tube, and extraction was repeated as indicated. Lysis solutions were then pooled and processed, as indicated in the original protocol. DNA was eluted in 200 µl of ultrapure H_2O.

TRI Reagent® – Samples were incubated at 70 °C in a thermomixer, set at 1,200 rpm, using 400 µl of TRI Reagent. After centrifugation, supernatant was collected, and extraction was repeated using an additional 400 µl of TRI Reagent. After precipitation and washing, DNA was resuspended in 200 µl of ultrapure H_2O.

Plant Extract-N-Amp™ – Samples were extracted with 200 µl of extraction solution, as indicated. After centrifugation (3 min 10,000 rpm), extraction solution was collected. Extraction was repeated with an additional 200 µl of extraction solution. Lysis solutions were pooled, and dilution buffer was added at 1:1 volume ratio. DNA was then recovered by sodium acetate–isopropyl alcohol precipitation [4], and resuspended in ultrapure H_2O. Amplifications of partial (500 nt) and whole (1,500 nt) sequence of small subunit ribosomal gene were performed using primer, as previously indicated [5]. All reactions were conducted in a final volume of 30 µl, using 10 pmol of each primer and 5 µl of extracted DNA. REDTaq® ReadyMix™ was used for partial fragment. Thermal profile was set with denaturation at 94 °C for 4 minutes, followed by 30 cycles (92 °C 20 sec, 50 °C 30 sec, 72 °C 45 sec), and a final elongation step of 5 minutes at 72 °C. The whole gene sequence was amplified using KlenTaq® DNA LA Polymerase. Thermal profile was set with initial denaturation at 94 °C for 4 minutes, followed by 35 cycles (92 °C 20 sec, 68 °C 3 min), and a final elongation step of 5 minutes at 68 °C.

Results and Discussion

DNA isolation protocols and PCR methods have allowed amplification of 16S gene from the bacterial community affecting cultural heritage (Figure 2 and Figure 3). Performance of PCR is highly affected by the initial sample composition. For example, DNA isolated from site A seemed to contain fewer inhibitors than DNA from site B, resulting in more efficient PCR reactions compared to DNA from site B (Figure 1, lanes 2, 4). PCR reaction of DNA, extracted from site A by the Extract-N-Amp™ procedure, provided an opalescent precipitate (Figure 2, top of lane 5) despite the high yield provided by the protocol. A simple alcohol precipitation easily removed these contaminants, allowing PCR amplification (Figure 2 and 3, lanes 7 and 8).

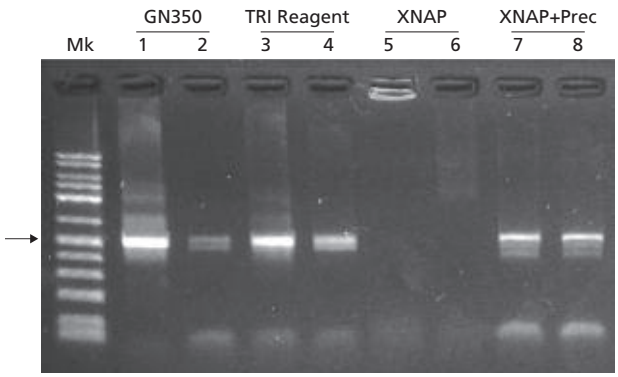

Figure 2. *Amplification of 500 bp fragment of 16S ribosomal gene by REDTaq™ ReadyMix™. Mk: Euroladder M, arrow 525 bp. Lanes 1, 3, 5, 7 archeological site A. Lanes 2, 4, 6, 8 archeological site B.*

In general, the amplification of DNA fragment of 1 Kb or more is difficult for this kind of matrix due to the presence of inhibitors or DNA sharing. The use of a more efficient and processive DNA polymerase compared to other native Taq polymerases, such as KlenTaq LA, has overcome this problem (Figure 3). Differences in PCR performance among samples were observed. Such differences may be attributed to co-extracted inhibitors or sharing of DNA in the sample B, as observed in the amplification of short 16S gene fragment.

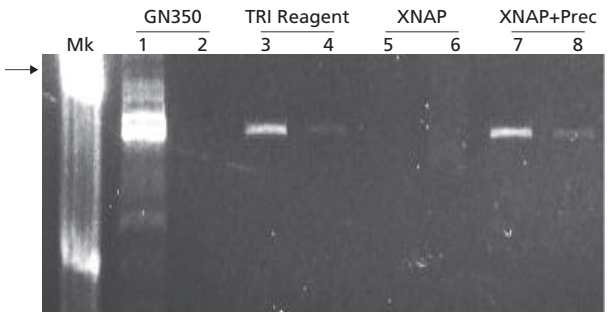

Figure 3. *Amplification of 1500 bp fragment of 16S ribosomal gene by KlenTaq LA™. Mk: Lambda/HindIII, arrow 2000 bp. Lanes 1, 3, 5, 7 archeological site A. Lanes 2, 4, 6, 8 archeological site B.*

Summary

These kits have provided rapid and simplified procedures for genomic DNA extraction directly from cultural heritage samples. They have resulted in peculiar features in terms of rapidity, yield, or purity of DNA. In our laboratories we usually work with DNA from an environmental matrix, traditionally considered difficult to analyze, such as soil, compost, sediments, and activated sludge. The choice of a more appropriate DNA extraction procedure should be tested for each of these samples. All of the kits that we tested, coupled with REDTaq™ ReadyMix™, have provided an effective tool of investigation in the heritage bacterial community (Figure 1). For a difficult analysis such as this, KlenTaq DNA LA Polymerase should be considered an effective tool for amplification of DNA. We use it for amplification of DNA from cultural or other environmental samples.

References

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Ordering Information

Cat. No.	Description	Unit
G2N10	GenElute™ Plant Genomic DNA Miniprep Kit (10 purifications)	1 kit
G2N70	GenElute™ Plant Genomic DNA Miniprep Kit (70 purifications)	1 kit
G2N350	GenElute™ Plant Genomic DNA Miniprep Kit (350 purifications)	1 kit
T9424	TRI Reagent® For processing tissues, cells cultured in monolayer or cell pellets	25 ml 100 ml 200 ml
93289	TRI Reagent® for DNA, RNA and protein isolation	25 ml 100 ml
T3809	TRI Reagent®, BD	25 ml 100 ml 200 ml
T3934	TRI Reagent®, LS	25 ml 100 ml 200 ml
XNAP2	Extract-N-Amp™ Plant PCR Kit (100 extractions, 100 amplifications)	1 kit
XNAP2E	Extract-N-Amp™ Plant PCR Kit (100 extractions, 500 amplifications)	1 kit
XNAR	Extract-N-Amp™ Plant PCR Kit (1,000 extractions, 1,000 amplifications)	1 kit
XNAP2RE	Extract-N-Amp™ Plant PCR Kit (1,000 extractions, 5,000 amplifications)	1 kit