Extremophiles (2008) 12:301–308
DOI 10.1007/s00792-007-0124-8

METHOD PAPER

Lysis efficiency of standard DNA extraction methods for *Halococcus* spp. in an organic rich environment

S. Leuko · F. Goh · R. Ibáñez-Peral · B. P. Burns · M. R. Walter · B. A. Neilan

Received: 24 August 2007 / Accepted: 5 November 2007 / Published online: 18 December 2007

Abstract The extraction of nucleic acids from a given environment marks a crucial and essential starting point in any molecular investigation. Members of *Halococcus* spp. are known for their rigid cell walls, and are thus difficult to lyse and could potentially be overlooked in an environment. Furthermore, the lack of a suitable lysis method hinders subsequent molecular analysis. The effects of six different DNA extraction methods were tested on *Halococcus hamelinensis*, *Halococcus saccharolyticus* and *Halobacterium salinarum* NRC-1 as well as on an organic rich, highly carbonated sediment from stromatolites spiked with *Halococcus hamelinensis*. The methods tested were based on physical disruption (boiling and freeze/thawing), chemical lysis (Triton X-100, potassium ethyl xanthogenate (XS) buffer and CTAB) and on enzymatic lysis (lysozyme). Results showed that boiling and freeze/thawing had little effect on the lysis of both *Halococcus* strains. Methods based on chemical lysis (Triton X-100, XS-buffer, and CTAB) showed the best results, however, Triton X-100 treatment failed to produce visible DNA fragments. Using a combination of bead beating, chemical lysis with lysozyme, and thermal shock, lysis of cells was achieved however DNA was badly sheared. Lysis of cells and DNA extraction of samples from spiked sediment proved to be difficult, with the XS-buffer method indicating the best results. This study provides an evaluation of six commonly used methods of cell lysis and DNA extraction of *Halococcus* spp., and the suitability of the resulting DNA for molecular analysis.

Keywords *Halococcus* · Cell lysis · XS-buffer · Organic rich environment · Stromatolites

Communicated by K. Horikoshi.

S. Leuko · R. Ibáñez-Peral · B. P. Burns · M. R. Walter · B. A. Neilan
Australian Centre for Astrobiology, Macquarie University, Sydney, NSW 2109, Australia

F. Goh · B. P. Burns · B. A. Neilan (✉)
Cyanobacteria and Astrobiology Research Lab, School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, NSW 2052, Australia
e-mail: b.neilan@unsw.edu.au

Introduction

In the past decade, applications of molecular approaches have provided unique insights into the uncultured microbial communities of environments as they avoid certain biases inherent in traditional culture-based microbiological methods (Miller et al. 1999). However, investigating diversity with non-culturing approaches, avoiding culture-dependent methods, can also result in new obstacles that can significantly bias any study (Miller et al. 1999; v. Wintzingerode et al. 1997).

During a molecular investigation of an environment, the lysis of all microorganisms within the given habitat is important and has to comply with two requirements: the effective lysis of cells, and the removal of any possible inhibitors for further analysis (e.g. humic acids). Furthermore, the resulting DNA should be intact and not sheared since strongly fragmented nucleic acids are sources of artifacts in PCR amplification and may contribute to the formation of chimeric PCR products (Liesack et al. 1991). Taking all these requirements into account, every PCR-based method will most likely be biased by an insufficient or preferential disruption of cells (v. Wintzingerode et al. 1997). This results in an underestimation of

the biodiversity present, and thus the choice of the optimal extraction method is critical.

Presently, many different DNA extraction methods exist (Leff et al. 1995; Ochsenreiter et al. 2002; Radax et al. 2001; Tillett and Neilan 2000), and are specifically designed for a particular environment and/or a particular family of microorganisms. However, each protocol usually includes one, two or all three of the following basic elements: physical disruption, chemical lysis and enzymatic lysis (Miller et al. 1999). Methods employing physical disruption techniques can be divided into four subgroups, that comprises freeze/thawing (Hugenholtz et al. 1998; Tsai and Olson 1991), grinding in liquid nitrogen (Zhou et al. 1996), ultrasonication (Gabig-Ciminska et al. 2005), and bead mill homogenization (Radax et al. 2001). A combination of freeze/thawing and bead mill homogenization is one of the most common techniques in use (Miller et al. 1999). Protocols belonging to the chemical lysis group can be further categorized into procedures that contain detergents, such as sodium dodecyl sulphate (Kuske et al. 1998; Ogram et al. 1987), potassium ethyl xanthogenate (XS) (Tillett and Neilan 2000) and cetyl trimethyl ammonium bromide (CTAB) (Wilson 1990), and procedures that contain various buffers (Miller et al. 1999). The enzymatic lysis employs specific enzymes for defined types of microorganisms, including lysozyme for Gram-negative bacteria, achromopeptidase for Gram-positive bacteria, and lyticase for fungal cells (Purdy 2005). A critical step following lysis is the removal of various biotic and abiotic components of environmental ecosystems, such as inorganic particles or organic matter. These materials can affect lysis efficiency and may interfere with subsequent DNA purification (Miller et al. 1999; v. Wintzingerode et al. 1997). In particular, the removal of humic acids is crucial, as these substances are known to interfere with and inhibit *Taq* DNA polymerase in PCR reactions (Smalla et al. 1993).

The focus of the present study was to elucidate the efficiency of six DNA extraction methods applied to halophilic archaea, in particular to representatives of *Halococcus* spp. Halophilic archaea typically thrive in high ionic environments such as solar salterns (Benlloch et al. 2001), the Dead Sea (Oren et al. 2006) and even ancient rock salt (Mormile et al. 2003; Stan-Lotter et al. 2002). Members of *Halococcus* spp. possess the ability to withstand low ionic environments and can remain viable at salinity values well below those that support growth (Oren 1994; Rodríguez-Valera et al. 1979). The main reason for this stability in hypoosmotic conditions is their rigid cell wall. In general, archaea lack murein, a peptidoglycan with numerous chemical variations that forms rigid cell walls in almost all taxa of bacteria with only a few exceptions (Kandler and König 1998). The cell wall of *Halococcus morrhuae* consists of a mixture of neutral and amino sugars, uronic acids and a particular aminuronic acid, gulosaminuronic acid (Kandler and König 1998).

Members of *Halococcus* spp. are very difficult to lyse, therefore we have attempted to optimize extraction methods so that this genus is subsequently amenable to molecular analysis. Although it is well known that lysozyme does not lyse *Halococcus* spp., previous work by Radax et al. (2001) indicated that a combination of lysozyme, SDS, bead beating and thermal shock was effective for the lysis of halophilic archaea. This genus is relevant as it is now being discovered in many interesting environments (e.g. stromatolites), and some members even possess novel metabolisms (Goh et al. 2006). We have chosen stromatolitic material for these experiments since recent publications have shown a broad diversity of halophilic archaea within these geological structures (Burns et al. 2004; Leuko et al. 2007; Papineau et al. 2005). We have optimized and evaluated several methods of cell lysis and DNA extraction using members of *Halococcus* spp. and for extraction of total DNA from modern stromatolites.

Materials and methods

Cultivation of strains

Samples of *Halococcus saccharolyticus* and *Halococcus hamelinensis* were cultivated in DSM97 medium (DasSarma et al. 1995) with an additional 7.23 g/l $MgCl_2{\cdot}6H_2O$ and 2.70 g/l $CaCl_2{\cdot}2H_2O$. Samples were incubated aerobically at 37°C with 160 rpm shaking until late exponential phase. *Halobacterium salinarum* NRC-1 was cultivated in ATCC 2185 medium and was incubated at the same conditions. To ensure accurate amounts of cells were employed and to enable each method of DNA extraction to be reliably compared, colony forming units (CFUs) were determined for each late exponential culture. Approximately 10^8 cells of a late exponential culture were centrifuged for 5 min at 5,000*g* and the supernatant was removed. Cell pellets were stored at −20°C until further use.

Stromatolite spiking

Halococcus hamelinensis, a novel halophilic archaeon isolated from modern stromatolites (mineralic microbial constructions), was used for ground stromatolite spiking experiments. Environmental stromatolite samples were obtained from Shark Bay at Telegraph Station (26°25′00” S, 114°13′05” E). Samples were homogenized with a mortar and pestle and sterilised by autoclaving twice at

121°C for 60 min (Zhou et al. 1996). Cells of *Hcc. hamelinensis* were washed three times with 4 M TN buffer (contained 4 M NaCl and 100 mM Tris, pH 7.4) and were mixed with the sterilised ground stromatolite to a final concentration of 10^6 and 10^4 cells/g ground stromatolite, respectively. A 0.5 g aliquot of ground stromatolite was used for each experiment. Unspiked ground stromatolite was used as a negative control for every extraction and subsequent PCR amplification.

DNA extraction methods

Extraction method A: boiling samples for 10 min

Pure cultures were resuspended in 200 µl dH_2O, vortexed and boiled for 10 min. Following this step, samples were centrifuged for 5 min at 5,000*g* and supernatant was transferred into a fresh tube. Ground stromatolitic samples were resuspended in 500 µl dH_2O and treated as stated above. DNA was purified as stated below.

Extraction method B: freeze/thawing cycles

Pure cultures were resuspended in 200 µl dH_2O and vortexed for 1 min. Samples were placed at −40°C for 15 min and then transferred to 70°C for 15 min. This procedure was repeated five times. Following these steps, samples were centrifuged for 5 min at 5,000*g* and the supernatant was transferred into a fresh tube. Ground stromatolitic samples were resuspended in 500 µl dH_2O and treated as stated above. DNA was purified as stated below.

Extraction method C: triton X-100

Pure cultures were resuspended in 40 µl 0.1% Triton X-100 and 10 µl 0.4 M NaCl. Samples were incubated for 5 min at 95°C and an additional 10 µl of 1 M Tris–HCl pH 7.4 was added. Ground stromatolitic samples were resuspended in 400 µl 0.1% Triton X-100 and 100 µl of 0.4 M NaCl and additional 100 µl of 1 M Tris–HCl pH 7.4 was added. Samples were centrifuged for 15 min at 12,000*g* and the supernatant was transferred into fresh tubes and DNA was purified as described below.

Extraction method D: potassium ethyl xanthogenate (XS) buffer

This method is based on the use of potassium ethyl xanthogenate followed by an incubation of the samples for 2 h at 65°C. Pure cultures were resuspended in 500 µl XS buffer containing 1% potassium ethyl xanthogenate, 20 mM EDTA, 1% SDS, 800 mM ammonium acetate and 100 mM Tris–HCl pH 7.4. Ground stromatolitic samples were resuspended in 1 ml of XS buffer. Samples were vortexed for 2 min and incubated for 2 h at 65°C and briefly mixed every half hour. Following incubation, cells were vortexed for 10 s and transferred on ice for 10 min. Samples were then centrifuged for 10 min at 12,000*g* and the supernatant was collected in a fresh tube and DNA was purified as described below.

Extraction method E: cetyl trimethyl ammonium bromide (CTAB)

Pure cultures, as well as ground stromatolitic samples, were resuspended in 576 µl TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA), 30 µl 10% SDS, 3 µl proteinase K (10 mg/ml) and 6 µl RNase (10 mg/ml). The solution was incubated for 3 h at 37°C with intermittent shaking (150 rpm). Following this step, 100 µl of 5 M NaCl and 80 µl of CTAB (4.1 g NaCl and 10 g CTAB dissolved in 100 ml dH_2O) were added to the solution and incubated for 20 min at 65°C. To remove the remaining CTAB and proteins from the solution, 1 V of Chloroform:Isoamylalcohol (24:1) was added, the solution gently inverted and centrifuged for 5 min at 12,000*g*. The aqueous layer was transferred into a fresh tube and DNA was further purified as described below.

Extraction method F: Combination of enzymatic (lysozyme), chemical (SDS), and physical lysis methods (bead beating and thermal shocks)

Pure cultures and ground stromatolitic samples were resuspended in 500 µl of TE buffer (100 mM Tris–HCl pH 8.0, 10 mM EDTA, 1% SDS) and 50 mg glass beads. This mixture was vortexed for 2 min after which 25 µl lysozyme (dissolved in 1% TE buffer) was added and the samples incubated for 1 h at 37°C. Following this step the samples were boiled for 10 min. Subsequently, the solution was vortexed for 2 min and 500 µl lysis buffer (4% SDS, 50 mM Tris–HCl, 100 mM EDTA) and 20 µl proteinase K (10 mg/ml) were added. Samples were vortexed and incubated for 1 h at 56°C. Finally, samples were freeze/thawed five times at −40°C and 70°C, respectively. Samples were centrifuged for 5 min at 5,000*g* and the supernatant was transferred into a fresh tube. DNA was purified as described below.

DNA purification

DNA extracted from pure cultures was purified using phenol–chloroform–isoamylalcohol (PCI) and isopropanol

precipitation as previously described by Sambrook et al. (1989). Purified DNA was resuspended in 100 µl dH_2O and stored at 4°C until further analysis. Quantities recovered were measured using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, DE, USA). For spiked ground stromatolitic samples, two different purification methods were evaluated: Method (I) the standard PCI and isopropanol precipitation (up to three times); and Method (II) an isopropanol precipitation step before the first PCI extraction, followed by the standard protocol. PCR reactions were performed using DNA recovered from spiked samples to evaluate the quality of the DNA.

Viability and cell morphology

Following every extraction method, changes to cell morphology as well as cell viability were investigated. Following cell disruption, the remaining cell pellet was resuspended in 500 µl 4 M TN buffer. Samples were washed three times with 1 ml of 4 M TN buffer and 50 µl aliquots were transferred into a fresh tube. Samples were stained with the LIVE/DEAD® BacLight™ Kit as previously described by Stan-Lotter et al. (2006). Cells were visualized using an epifluorescence microscope (Olympus BH2-RFC) fitted with a mercury burner of broad band excitation. Images were collected using a Nikon digital camera DXM1200F and Nikon ACT-1 version 2.62 software. To confirm the results observed with the LIVE/DEAD Kit, representative samples of each extraction were evaluated by determining colony forming units (CFUs).

PCR conditions and optimisation

Recovered and purified DNA from spiked ground stromatolitic samples (extractions were performed in triplicates) was used as templates for 16S rRNA gene amplification to evaluate the quality of the recovered DNA. Primers A21F and A958R were employed (DeLong 1992) and 16S rRNA genes were amplified using the following conditions: initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 45 s with a final elongation step at 72°C for 3 min. PCR was performed in a 20 µl reaction volume containing 2 µl 10× *Taq* polymerase buffer, 0.2 mM dNTPs, 10 pmol of each primer, 4 mM $MgCl_2$ and 0.4 units of *Taq* polymerase. Following amplification, products were analysed on a 1% agarose gel stained with ethidium bromide. To test extracted and purified DNA samples for the presence of PCR inhibitors, extraction samples were spiked with ~20 ng purified DNA extracted from *Hcc. hamelinensis*. Furthermore, extracted DNA was diluted (1:10, 1:20, 1:50, and 1:100) with dH_2O to evaluate beneficial effects.

Results

Effect of lysis methods using pure cultures

The lysis efficiency of six extraction methods was tested using *Hcc. hamelinensis* and *Hcc. saccharolyticus* as representative halococci strains and the results are given in Table 1. *Hbt. salinarum* NRC-1 was used as a positive control to evaluate the reproducibility of each extraction method, as *Hbt. salinarum* NRC1 is easy to lyse. The least efficient method was freeze/thawing (Extraction method B) with the smallest amount of DNA recovered. A similar low recovery rate was observed for boiling the samples (Extraction method A). Both methods also failed to produce visible DNA bands on a 1% agarose gel following extraction (data not shown). Treatment with Triton X-100 (Extraction method C) showed a slight increase in DNA yield for *Hcc. hamelinensis*, compared to boiling and freeze/thawing, but showed similar amounts for *Hcc. saccharolyticus* compared to freeze/thawing and boiling (Table 1).

The quantity of DNA recovered using chemical lysis was much higher compared to physical disruption, with the combination of enzymatic, chemical and physical disruption method (Extraction method F) resulting in the highest yields. However, employing extraction method F resulted in badly sheared DNA (Fig. 1b), likely to be caused by bead beating the sample for 2 min. Although the shearing of *Hbt. salinarum* NRC-1 does not appear to be as high when compared to the other samples, the amount of DNA recovered was much higher and therefore still resulted in a considerable amount of intact DNA. However, when compared to the XS-buffer extraction (Fig. 1a), the strong increase in sheared DNA using method F can be seen. The XS-buffer method was shown to be an efficient and fast method to lyse *Halococcus* spp., with high amounts of DNA recovered and low levels of sheared DNA (Fig. 1a). A similar result was obtained for the CTAB method, with comparable quantity and quality of DNA recovered. Following every extraction we evaluated the survival and cell morphology of the cells using the LIVE/DEAD® *Bac*Light™ Kit and by calculating CFUs. All treatments resulted in dead cells (indicated by red fluorescence stain) with no green fluorescent staining observed (Fig. 2). However, the cell morphology did not change for *Hcc. hamelinensis* and *Hcc. saccharolyticus* following the tested methods, with the typical coccoid structure still visible (Fig. 2). *Hbt. salinarum* NRC-1, however, was fully lysed following every treatment with no cell structures visible under the microscope. In accordance with this result, no

Table 1 Comparison of DNA yield from pure cultures, subjected to different extraction methods

Treatment	*Hcc. saccarolyticus*	*Hcc. hamelinensis*	*Hbt. salinarum* NRC-1
(A) Boiling	96 (±31%)	86 (±13%)	239 (±20%)
(B) Freeze/thawing	71.6 (±10.2%)	46 (±8%)	347 (±36%)
(C) Triton X-100	90 (±13.2%)	119 (±14%)	353 (±30%)
(D) XS–buffer	188 (±29.1%)	209 (±37%)	400 (±36%)
(E) Lysozyme	242.7 (±3.3%)	296.3 (±12.4%)	449.1 (±15.4%)
(F) CTAB	203.5 (±30%)	193.7 (±21.1%)	378 (±16.9%)

DNA yields were measured using the Nanodrop ND-1000 spectrophotometer and the average yield of at least triplicate DNA extractions is given in ng/µl. Standard deviation is indicated in brackets

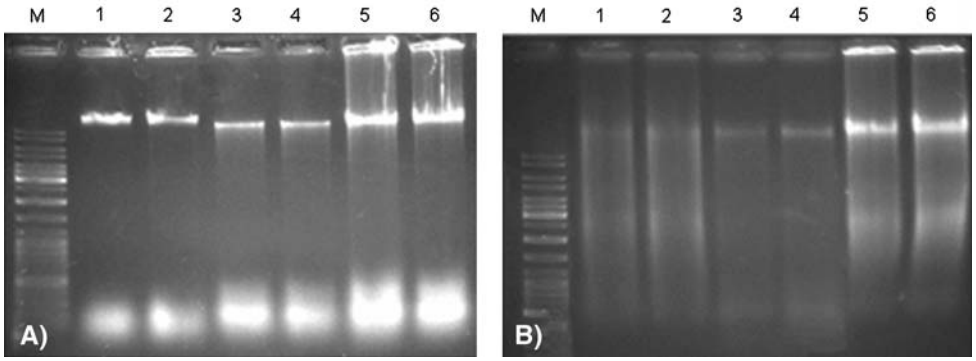

Fig. 1 Agarose gel electrophoresis of total DNA extraction from *Hcc. saccharolyticus*, *Hcc. hamelinensis* and *Hbt. salinarum* NRC-1 using (**a**) XS-buffer and (**b**) lysozyme. *Lane M*: Molecular marker; *lanes 1 and 2* DNA isolated from *Hcc. saccharolyticus*; *lanes 3 and 4* DNA isolated from *Hcc hamelinensis*; *lanes 5 and 6* DNA isolated from *Hbt. salinarum* NRC-1

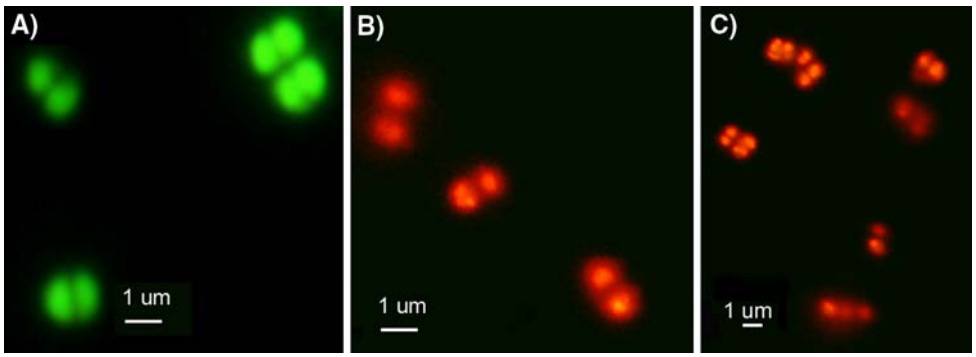

Fig. 2 Picture (**a**) shows a representative sample of *Hcc. hamelinensis* stained with the LIVE/DEAD® BacLight Kit without treatment. In comparison, cells of *Hcc. saccharolyticus* (**b**) and *Hcc. hamelinensis* (**c**) stained with the LIVE/DEAD® BacLight Kit following extraction using the XS-buffer method. Intact cell wall can be seen, however cells are biologically inactive as indicated by the red fluorescence

colony forming units were observed following each extraction method.

Efficiency of lysis methods on seeded sediment

Stromatolitic samples were sterilised and spiked with a known concentration of *Hcc. hamelinensis* and the six previously tested methods were used to extract DNA from these samples. Autoclaved, unspiked stromatolite samples were used as a negative control. None of these methods could recover visible quantities of DNA using samples spiked with 10^4 cell/g, nor was it possible to obtain any PCR amplification following several steps of extraction and purification. For stromatolitic material spiked with 10^6 cells/g the XS-buffer method proved again to be the most efficient method, producing a clear genomic DNA band following extraction (Fig. 3, lane 6). Extraction

using CTAB was the only other method resulting in a visible band after gel electrophoresis (Fig. 3, lane 7). Every other method failed to produce a visible band of genomic DNA, and was not successful in PCR amplifications.

DNA purification and PCR efficiency

Following every extraction a PCR was performed to test the purity of the recovered DNA. Although three consecutive PCI and isopropanol extractions were performed [Method (I)] for all the extraction procedures, it did not result in sufficiently pure DNA suitable for further investigations. None of the resulting DNA samples allowed amplification of a PCR product. Spiking the samples with a known amount of DNA or dilutions of the obtained DNA used as a template also did not result in a PCR product. Purification and precipitation employing method (II) was more efficient than method (I) with a faint PCR product visible following two PCI extractions and isopropanol precipitation using DNA extracted with the XS-buffer method (Fig. 4a). Every other method failed to produce suitable DNA for PCR amplification following three purification and precipitation steps employing method (II). Based on these results, the XS-buffer DNA extraction method was used for further PCR optimisation tests. Although a PCR product was obtained from XS-buffer extracted DNA purified with method (II), this PCR product was not suitably pure for further work (e.g. sequencing, clone library construction). Testing several dilutions of the DNA indicated that a 1:100 dilution removed most of the inhibitors and also contained sufficient DNA for a successful PCR reaction (Fig. 4b).

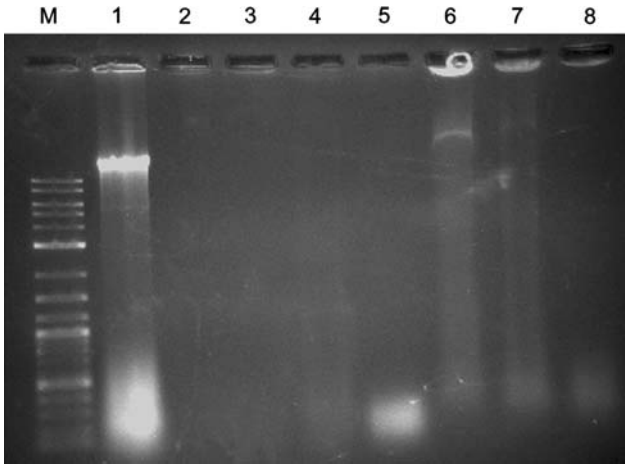

Fig. 3 Agarose gel electrophoresis of total DNA extracted from stromatolitic soil by different treatments. *Lanes M* Molecular marker; *lane 1* pure-culture DNA from *Hcc. hamelinensis*; *lane 2* un-spiked ground stromatolite; *lane 3* spiked ground stromatolite extracted by boiling; *lane 4* spiked ground stromatolite extracted with freeze/thawing; *lane 5* spiked ground stromatolite extracted with Triton X-100; *lane 6* spiked ground stromatolite extracted with XS-buffer; *lane 7* spiked ground stromatolite extracted with CTAB; *lane 8* spiked ground stromatolite extracted with lysozyme

Discussion

Extracting DNA from a given environment has been the focus of many previous studies as it marks a crucial step for any kind of further molecular investigations (Martin-Laurent et al. 2001; Miller et al. 1999; Purdy 2005). It is therefore not surprising that there are numerous DNA extraction methods available. The range of methods available reflects the heterogeneity of environmental samples in respect to soil properties such as pH, clay, silt content, and organic matter (Fortin et al. 2004). Even

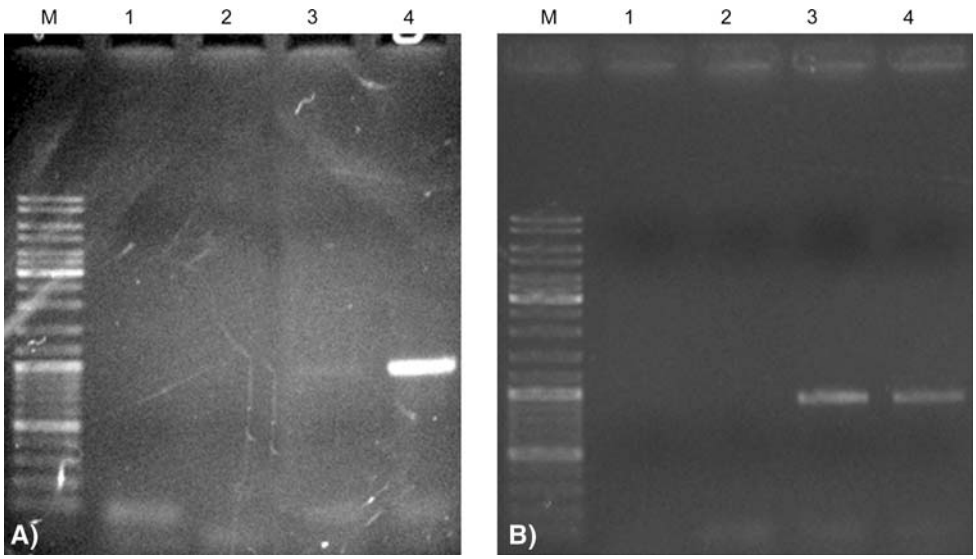

Fig. 4 PCR results of 16S rRNA gene amplification applying undiluted and diluted DNA extracted from spiked ground stromatolitic samples using the XS-buffer method. **a** shows results for the undiluted DNA template; **b** shows the results for a 1:100 diluted DNA template. Lanes: *M* marker lane; *lane 1* PCR negative control; *lane 2* un-spiked stromatolitic soil; *lane 3* DNA template extracted with XS-buffer (a: undiluted; b: 1:100 dilution); *lane 4* positive control (~20 ng DNA from *Hcc. hamelinensis*)

though every method follows a clear protocol, the results often vary, due to the fact that there are many steps involved which can not be standardized, e.g. removing the aqueous phase following PCI extraction. During the course of investigations we encountered this problem in particular when trying to determine the quantity of DNA recovered (Table 1). While the extractions were repeated numerous times, the amount recovered varied strongly within a method, resulting in a high standard deviation. Despite the high standard deviations, the results allowed us to determine the efficiency of the cell lysis protocol.

The focus of this study was to test six lysis methods on two representatives of *Halococcus* spp. This genus is known for its tough and rigid cell wall (Kandler and König 1998). Methods solely based on physical disruption of cells (boiling and freeze/thawing) have been previously used to isolate DNA from microorganism such as yeast (Harju et al. 2004) and *Mycobacterium avium* (O'Mahony and Hill 2004), as well as from hypersaline environments (Antón et al. 2000). These methods, however, proved to be ineffective in lysing pure cultures of *Hcc. hamelinensis* and *Hcc. saccharolyticus* in the present study. These methods also failed to both extract visible amounts of DNA and provide sufficient DNA for PCR amplification from stromatolite samples.

Chemical lysis showed the best results with respect to cell lysis and DNA purity. Although all four chemical methods tested had some beneficial effects, the methods differed with respect to time consumption, DNA quality and quantity recovered. The method employing a combination of enzymatic lysis (lysozyme), chemical lysis (SDS) and physical disruption (bead beating and thermal shocks) resulted in the greatest DNA recovery, but sheared the DNA badly during the course of extraction and was also the most labour intensive. The CTAB and XS-buffer methods, both recovered less DNA compared to the combination of enzymatic, chemical lysis and physical disruption procedure. However, the CTAB and XS-buffer methods lyse the cells more gently, resulting in very little sheared DNA. Both methods recovered similar amounts of DNA with similar quality. Comparing both CTAB and XS-buffer methods, the XS-buffer has the advantage of only using one buffer and one incubation step compared to two incubation steps and more reagents required for the CTAB. The Triton X-100 method is less labour intensive and uses few reagents, however, only very low quantities of DNA could be recovered from pure cultures with no bands visible on an agarose gel (data not shown).

An important issue in current estimates of bacterial/archaeal diversity is related with the sensitivity of post DNA extraction PCR-based procedures (Luna et al. 2006). Several previous studies emphasise that the presence of Ca^{2+} ions interferes with Mg^{2+}, thus decreasing the PCR efficiency (Bickley et al. 1996; Wilson 1997). Stromatolites, largely composed of calcium carbonate in combination with organic material, present a difficult environmental sample for a successful DNA extraction and subsequent PCR reaction. None of the tested extraction methods completely removed all of the contaminants from these samples using one standard purification step. Additional purification steps after extraction using PCI were required to reduce polysaccharide and humic acid contaminations, respectively (Malik et al. 1994; Miller et al. 1999). During the course of this study we found that, using the standard purification method (I), three consecutive purification steps employing PCI and isopropanol did not result in sufficiently clean DNA for subsequent PCR amplification. However, by conducting an isopropanol step before the first PCI step [method (II)], only two purifications were necessary.

By applying this purification step (method II) to all six extraction methods we found the best results for this environment were obtained by using the XS-buffer method. All other procedures failed to provide DNA suitable for PCR under experimental conditions. Although PCR amplification following XS-buffer extraction could be observed, the amplification efficiency was weak suggesting that inhibitor may still have been present. To overcome this problem, diluting the samples provided a rapid and straightforward way of permitting amplification (Wilson 1997). Using a 1:100 dilution of DNA, we were able to amplify the 16S rRNA gene (Fig. 4), however 1:20 and 1:50 dilutions did not result in PCR amplification. Although diluting the recovered DNA resulted in an improved amplification, care must be taken with dilutions since the DNA concentrations will also ultimately influence the PCR efficiency (v. Wintzingerode et al. 1997). We conclude that the XS-buffer method is a fast and reliable method to lyse members of *Halococcus* spp. However, further investigations are necessary to develop a method which lyses *Halococcus* spp. completely without shearing the DNA.

Acknowledgements This research was funded by the Australian Research Council and an International Postgraduate Research Scholarship from Macquarie University, Australia. We would also like to thank two anonymous reviewers for very useful comments which have improved this manuscript.

References

Antón J, Rosselló-Mora R, Rodríguez-Valera F, Amann R (2000) Extremely halophilic bacteria in crystallizer ponds from solar salterns. Appl Environ Microbiol 66:3052–3057

Benlloch S, Acinas SG, Antón J, López-López A, Luz SP, Rodríguez-Valera F (2001) Archaeal biodiversity in crystallizer ponds from a solar saltern: culture versus PCR. Microb Ecol 41:12–19

Bickley J, Short JK, McDowell DG, Parkes HC (1996) Polymerase chain reaction (PCR) detection of *Listeria monocytogenes* in diluted milk and reversal of PCR inhibition caused by calcium ions. Lett Appl Microbiol 22:153–158

Burns BP, Goh F, Allen MA, Neilan BA (2004) Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia. Environ Microbiol 6:1096–1101

DasSarma S, Fleischmann EM, Rodríguez-Valera F (1995) Halophiles. In: Robb FT (ed) Archaea: a laboratory manual. CSHL Press, Cold Spring Harbor, USA, pp 225–230

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689

Fortin N, Beaumier D, Lee K, Greer CW (2004) Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. J Microbiol Meth 56:181–191

Gabig-Ciminska M, Liu Y, Enfors SO (2005) Gene-based identification of bacterial colonies with an electric chip. Anal Biochem 345:270–276

Goh F, Leuko S, Allen MA, Bowman JP, Kamekura M, Neilan BA, Burns BP (2006) *Halococcus hamelinensis* sp. nov., a novel halophilic archaeon isolated from stromatolites in Shark Bay, Australia. Int J Syst Evol Microbiol 56:1323–1329

Harju S, Fedosyuk H, Peterson KR (2004) Rapid isolation of yeast genomic DNA: bust'n grab. BMC Biotechnol 4:8

Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765–4774

Kandler O, König H (1998) Cell wall polymers in Archaea (Archaebacteria). Cell Mol Life Sci 54:305–308

Kuske CR, Banton KL, Adorada DL, Stark PC, Hill KK, Jackson PJ (1998) Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. Appl Environ Microbiol 64:2463–2472

Leff LG, Dana JR, McArthur JV, Shimkets LJ (1995) Comparison of methods of DNA extraction from stream sediments. Appl Environ Microbiol 61:1141–1143

Leuko S, Goh F, Allen MA, Burns BP, Walter MR, Neilan BA (2007) Analysis of intergenic spacer region length polymorphisms to investigate the halophilic archaeal diversity of stromatolites and microbial mats. Extremophiles 11:203–210

Liesack W, Weyland H, Stackebrandt E (1991) Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic Bacteria. Microb Ecol 21:191–198

Luna GM, Dell'Anno A, Danovaro R (2006) DNA extraction procedure: a critical issue for bacterial diversity assessment in marine sediments. Environ Microbiol 8:308–320

Malik M, Kain J, Pettigrew C, Ogram A (1994) Purification and molecular analysis of microbial DNA from compost. J Microbiol Meth 20:183–196

Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G, Catroux G (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. Appl Environ Microbiol 67:2354–2359

Miller DN, Bryant JE, Madsen EL, Ghiorse WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65:4715–4724

Mormile MR, Biesen MA, Gutierrez MC, Ventosa A, Pavlovich JB, Onstott TC, Fredrickson JK (2003) Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions. Environ Microbiol 5:1094–1102

O'Mahony J, Hill C (2004) Rapid real-time PCR assay for detection and quantitation of *Mycobacterium avium* subsp. *paratuberculosis* DNA in artificially contaminated milk. Appl Environ Microbiol 70:4561–4568

Ochsenreiter T, Pfeifer F, Schleper C (2002) Diversity of Archaea in hypersaline environments characterized by molecular-phylogenetic and cultivation studies. Extremophiles 6:267–274

Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. J Microbiol Meth 7:57–66

Oren A (1994) The ecology of the extremely halophilic archaea. FEMS Microbiol Rev 13:415–440

Oren A, Gavrieli I, Gavrieli J, Kohen M, Lati J, Aharoni M (2006) Microbial communities in the Dead Sea—past, present and future. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds) Adaptation to life at high salt concentrations in Archaea, Bacteria and Eukarya. Springer, The Netherlands, pp 29–40

Papineau D, Walker JJ, Mojzsis SJ, Pace NR (2005) Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl Environ Microbiol 71:4822–4832

Purdy KJ (2005) Nucleic acid recovery from complex environmental samples. In: Leadbetter JR (ed) Methods of enzymology, vol 397. Elsevier, New York, pp 271–292

Radax C, Gruber C, Stan-Lotter H (2001) Novel haloarchaeal 16S rRNA gene sequences from alpine permo-triassic rock salt. Extremophiles 5:221–228

Rodríguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A (1979) Isolation of extreme halophiles from seawater. Appl Environ Microbiol 38:164–165

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Habor Laboratory Press, Cold Spring Harbor, NY

Smalla K, Cresswell N, Mendonca-Hagler C, Wolters A, van Elsas JD (1993) Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. J Appl Bacteriol 74:78–85

Stan-Lotter H, Pfaffenhuemer M, Legat A, Busse HJ, Radax C, Gruber C (2002) *Halococcus dombrowskii* sp. nov., an archaeal isolate from a Permian alpine salt deposit. Int J Syst Evol Microbiol 52:1807–1814

Stan-Lotter H, Leuko S, Legat A, Fendrihan S (2006) The assessment of the viability of halophilic microorganisms In: Rainy FA, Oren A (eds) Methods in microbiology, vol 35. Academic, London, pp 569–584

Tillett D, Neilan BA (2000) Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J Phycol 36:251–258

Tsai YL, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol 57:1070–1074

Wilson K (1990) Preparation of genomic DNA from bacteria In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) Current protocols in molecular biology. Wiley, New York, pp 2.4.1–2.4.2

Wilson IG (1997) Inhibition and facilitation of nucleic acid amplification. Appl Environ Microbiol 63:3741–3751

v. Wintzingerode F, Göbel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229

Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316–322