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A procedure for separate recovery of extra- and intracellular DNA from a single marine sediment sample

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ABSTRACT

Extracellular DNA (eDNA) is a ubiquitous biological compound in aquatic sediment and soil. Previous studies suggested that eDNA plays an important role in biogeochemical element cycling, horizontal gene transfer and stabilization of biofilm structures. Previous methods for eDNA extraction were either not suitable for oligotrophic sediments or only allowed quantification but no genetic analyses. Our procedure is based on cell detachment and eDNA liberation from sediment particles by sequential washing with an alkaline sodium phosphate buffer followed by a separation of cells and eDNA. The separated eDNA is then bound onto silica particles and purified, whereas the intracellular DNA from the separated cells is extracted using a commercial kit. The method provides extra- and intracellular DNA of high purity that is suitable for downstream applications like PCR. Extracellular DNA was extracted from organic-rich shallow sediment of the Baltic Sea, glacially influenced sediment of the Barents Sea and from the oligotrophic South Pacific Gyre. The eDNA concentration in these samples varied from 23 to 626 ng g^{-1} wet weight sediment. A number of experiments were performed to verify each processing step. Although extraction efficiency is higher than other published methods, it is not fully quantitative.

1. Introduction

Extracellular DNA (eDNA) is a major part of the total DNA pool in marine sediments and soils (Ceccherini et al., 2009; Corinaldesi et al., 2011, 2005; Dell' Anno and Danovaro, 2005). There are multiple sources for eDNA, cell death and subsequently cell lysis caused by physical or spontaneous damage of the prokaryotic and eukaryotic cell wall, pathogen-induced cell lysis, active extrusion and necrosis being the major processes (Corinaldesi et al., 2014; Levy-Booth et al., 2007; Lorenz and Wackernagel, 1994; Paget and Simonet, 1994). Also, eDNA might play an important role in biogeochemical element cycling (Dell' Anno and Danovaro, 2005), and horizontal gene transfer (Stewart and Sinigalliano, 1990). Das et al. (2010) described in detail the function and importance of eDNA for bacterial adhesion and aggregation on surfaces. Since DNA is also important for the stabilization of biofilm structures (Bockelmann et al., 2006; Dominiak et al., 2010; Molin and Tolker-Nielsen, 2003), biofilms might contribute to the eDNA pool as well.

The persistence of extracellular, high-molecular-weight DNA in sediment depends on several factors, e.g., mineralogy, ionic composition and pH of the pore water (Ogram et al., 1987, 1988; Poinar et al., 1996). Temperature might also affect the rate of biodegradation of eDNA, as enzyme activity is positively correlated with temperature (Lindahl, 1993). An important factor for the persistence of a DNA molecule in sediment is adsorption onto sediment particles (Paget et al., 1992). DNA attached to mineral surfaces has shown to be less bioavailable and is protected from degradation. Theoretical considerations and empirical studies suggested a maximal DNA persistence of 50 ka to 1 Ma (Hebsgaard et al., 2005). Extracellular DNA might include sequence information of ancient microbial and eukaryotic organisms (Pietramellara et al., 2008). Studies on ancient DNA provide insight into ancient life forms and environmental conditions (Hadly et al., 2004). Ancient DNA found in such old environments has been referred to as 'fossil DNA' or 'Paleome' (Bada et al., 1999; Coolen and Overmann, 1998; Inagaki et al., 2005).

Despite major methodological advances, analysis of DNA from sediment is still technically challenging, not just because of the co-elution of inhibitory substances, but also due to the co-elution of eDNA, which potentially leads to an overestimate of the actual diversity (Lombard et al., 2011; Luna et al., 2006). Thus, molecular biological analyses of DNA, extracted without separation of intracellular DNA (iDNA) and eDNA, might not just reflect the actual living microbial community, but also the Paleome.

Different eDNA extraction procedures for marine sediments and soils have been published (Ceccherini et al., 2009; Corinaldesi et al., 2005; Dell' Anno and Corinaldesi, 2004; Luna et al., 2006; Ogram et al., 1988), but they are rarely applied, mainly due to the increase in processing time.

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The modified procedure described here is partially based on previously published extraction protocols. In general the extraction procedure includes the detachment of microbial cells and eDNA from sediment particles by several washing and centrifugation steps in an alkaline sodium phosphate (NaP) buffer followed by binding the eDNA onto silica particles.

Washing eDNA off the sediment with a sodium phosphate (NaP) buffer was introduced by Ogram et al. (1987) and further refined by Corinaldesi et al. (2005), who used a subsequent cetyltrimethyl-ammoniumbromid (CTAB)- based DNA precipitation step to recover and purify the eDNA. Instead of using the CTAB-based DNA precipitation we used silica particles (Boom et al., 1990) to extract and purify the eDNA.

These major modifications allow the extraction and quantification of extremely small amounts of eDNA in sediment samples. Further modifications of the protocol were made to increase eDNA recovery rates without artificially increasing the eDNA pool by lysing intact cells.

The aim of the study was to develop and evaluate a DNA extraction procedure for iDNA and eDNA that is also suitable for oligotrophic sediments with low cell numbers and low amounts of eDNA. Several tests were performed to assess the efficiency of each extraction step. Finally, three rather different sediments were analyzed, organic-rich Baltic Sea, organic-poor Barents Sea and extremely oligotrophic South Pacific Gyre (SPG) sediment. We compared the modified protocol to published cetyltrimethyl-ammoniumbromid (CTAB) and ethanol-based procedures (Corinaldesi et al., 2005) and a commercial available DNA purification kit (MoBio, UltraClean15). For the extraction of iDNA a commercial bead beating kit was used instead of a phenol-chloroform extraction. The extracted DNA fractions were analyzed by agarose gel-electrophoresis and 16S rRNA Polymerase Chain Reaction (PCR).

2. Materials and Methods

2.1. Samples

The characteristics of all samples are presented in Table 2. Baltic Sea samples were taken from the Eckernförde Bay with a miniature multicorer in July 2010. Subsamples were stored in gas-tight plastic bags. For further sampling details see Bertics et al. (2012). Barents Sea samples were taken in November 2009 with a 2 m long gravity corer, immediately sectioned into 10 cm whole round cores and stored in sterile gas-tight aluminum-coated plastic bags. Details on the sampling are given by Nickel et al. (2012). South Pacific Gyre sediment was collected on IODP Exp 329 (Site U-1371) in 2011. Samples were taken with sterile cut-off 20 mL syringes and stored in gas-tight plastic bags. Additionally it should be mentioned that in contrast to the Baltic Sea and Barents Sea sediment, the analyzed SPG sediment is oxic (D' Hondt et al., 2009). After retrieval all samples were frozen to −20 °C and stored at this temperature until immediately prior to analysis.

2.2. Preparation of reagents

2.2.1. Several reagents are necessary for the extraction procedure:

Sodium phosphate (NaP) buffer: Na_2HPO_4 and NaH_2PO_4 (0.12 M Na_2HPO_4, 2% NaCl, pH 8, Ogram et al., 1987). *Polyvinylpolypyrrolidon* (*PVPP*, Sigma-Aldrich order No. 77627, preparation according to Evans et al., 1972). *Guanidine hydrochloride (GuaHCl)* (6 M GuHCl in TE buffer, pH 6.7, 10 mM TRIS HCl, 1 mM EDTA). *Washing buffer*: 55% EtOH, 70 mM NaCl, 10 mM Tris, 2.6 mM EDTA

2.2.2. Preparation of the silica suspension

The silica particles (Sigma-Aldrich, SiO_2, 0.5–10 μm, order No. S5631) were heated to 450 °C for 5 h to remove all traces of organic carbon. The size fractionation was performed according to Boom et al. (1990). Briefly, silica particles were suspended in 500 mL sterile (0.2 μm filtered and autoclaved) demineralized water in a glass cylinder (height of water column, 27.5 cm; width, 5 cm) and left for 24 h at room temperature. A 430 mL portion of the supernatant was carefully siphoned off and discarded. The remaining 70 mL were brought back to a total volume of 500 mL with sterile demineralized water, and the silica pellet was suspended by vigorous shaking. After another 5 h of sedimentation, 440 mL of the supernatant was siphoned off and discarded. To adjust the remaining 60 mL of silica suspension to pH 2, 600 μl of HCl (32%, wt/vol) were added. The resulting suspension of coarse silica was adjusted to a density of 1.3 g/mL using sterile diethylpyrocarbonate (DEPC) treated water (Carl Roth, order No. T143.3). The suspension was aliquoted in 2 mL vials and can be stored for up to one month at 5 °C in the dark. Just before use the silica suspension was centrifuged (12,000 ×*g*, 20 s), the supernatant (acidified DEPC treated water, pH 2) was decanted off and replaced by an equal volume of non-acidified DEPC treated water (pH 7).

2.3. Preparation of eDNA standards

Baltic Sea sediment was used to prepare an eDNA stock solution. Only one washing cycle with NaP buffer was performed in order to avoid excessive dilution of the stock solution, because the eDNA concentration in subsequent washing cycles is considerably lower. Since RNA might influence the DNA quantification, a RNAse (Sigma-Aldrich, Type I-A, order No. R6513) treatment was carried out according to the manufacturer's recommendations. The eDNA stock solution was diluted with NaP buffer to the desired eDNA concentration of 0.002, 0.02, 0.2, 2 and 20 ng mL^{-1}.

2.4. Extraction of intra- and extracellular DNA

A flowchart of the complete procedure is given in Fig. 1.

2.4.1. Preparation of slurry

All preparations of slurry were performed at least in triplicate to check for variability in the extraction efficiency and to counter inhomogeneity in the sediment samples. Small pieces (ca. 2 g) of frozen sediment were placed in a sterile 15 mL polypropylene (PP) centrifugation vial and slowly thawed on ice. 4–8 mL of cold (4 °C) NaP buffer and 0.5 g PVPP were added to the thawed sediment. PVPP is used to bind

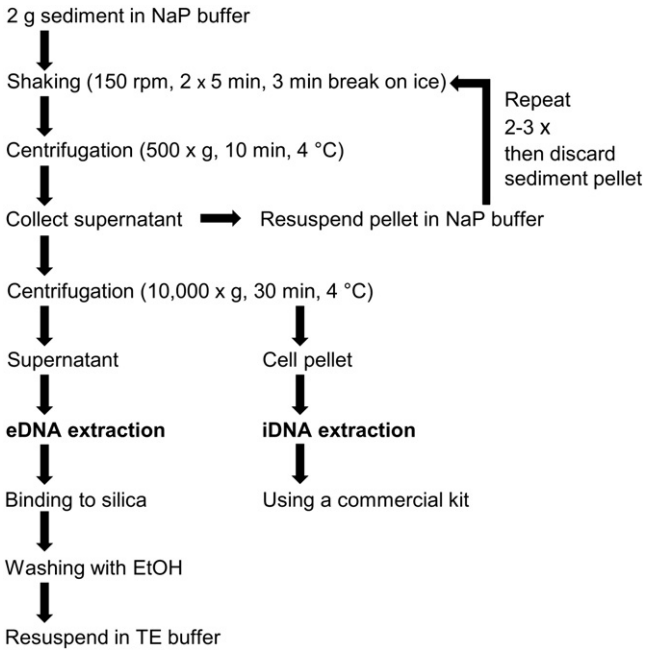

Fig. 1. Sequential extraction procedure for extracellular and intracellular DNA.