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Conference Abstract

Optimized protocol proposal to extract eDNA from oligotrophic and degraded water samples

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Abstract

Aquifer represents an oligotrophic environment that sustains a relatively small amount of microbial cells, mostly non-culturable. Due to this dominance of unculturable microorganisms in natural ecosystems, studying microbial communities and their functionality should include culture-independent approaches based on molecular techniques using DNA analysis (Purswani et al. 2011). For practical reasons, aquifer routine analyses focus on groundwater samples, while solid aquifer samples are typically not included (Ritalahti et al. 2010). The amount of groundwater collected, together with the types and concentrations of inhibitory compounds if present, determine the abundance of the target biomarker(s) available for subsequent analyses. Hence, filtering large volumes of groundwater seems beneficial, but for practical purposes, 0.5–2 L of water are typically collected, depending on groundwater characteristics (Ritalahti et al. 2010).

In this work, environmental DNA (eDNA) was extracted from groundwater samples, filtering three different initial volumes (1000 ml, 500 ml, and 250 ml) of water samples, using 0.22 µm membranes. Also, two DNA extraction commercial kits were tested, DNeasy PowerWater Kit and DNeasy PowerSoil kit (Qiagen, Germantown, MD) specific for water samples and solid matrix, respectively.

For DNeasy PowerWater Kit the standard protocol was carried out, whereas a modified protocol equipped with the Inhibitor Removal Technology® (IRT) was selected for testing DNeasy PowerSoil kit. In order to minimize operator bias, both the protocols were made semi-automated by using a QIAcube provided by Qiagen for genomic DNA extraction. Additional steps to the PowerSoil IRT protocol were performed for optimizing chemical and mechanical cell lysis processes and facilitating the sample dispersion into the buffer solutions. eDNA was verified via electrophoresis and quantified fluorometrically. eDNA extracted from 250 ml of groundwater sample using the DNeasy PowerSoil kit with modified IRT protocol was also tested in downstream applications, including Polymerase Chain Reactions (PCRs) with specific primer pairs for the identification of microbial targets.

Results suggest that the PowerSoil modified IRT protocol was the best performing one, allowing a higher eDNA yield from all the water sample volumes tested. In addition, plotting on a graph eDNA concentration values against sample volumes filtered, the yield of PowerSoil modified IRT protocol appeared more similar to an ideal direct proportionality than the yield of PowerWater standard protocol. eDNA quality was suitable for PCR analyses and the identification of bacterial targets, including bacterial subgroups (α and β-proteobacteria) and single species of interest, such as *Shewanella oneidensis* capable of hexavalent chromium reduction (Tumolo et al. 2020). Under these good performances, the PowerSoil optimized IRT protocol was also applied in a further experiment about bioremediation to extract eDNA from 50 ml of water spiked with 1000 µg/l of hexavalent chromium. The resulting genomic material was successfully used in quantitative PCR (qPCR) assays for monitoring the relative abundance of *Shewanella oneidensis* during the bioremediation process, allowing to highlight the hexavalent chromium inhibitor effect on the selected microbial target (Ancona et al. 2020).

Downstream applications of eDNA obtained using DNeasy PowerSoil kit resulted in positive outcomes for both the experiments previously described. In light of this, it is possible to conclude that this kit combined with the protocol adjustments proposed in this work, can be a performing tool for eDNA extraction, also from small amounts of water sample collected from oligotrophic or degraded environments.

Further investigations will be oriented to optimize eDNA extraction from the aquifer solid portion characterized by few nutrients and microbial cells to better understand how microbial populations can distribute themselves between solid and aqueous phases.

Keywords

eDNA, extraction, protocol, aquifer, groundwater, oligotrophic environment

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Conflicts of interest

The authors declare no conflict of interest.

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