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A hot-alkaline DNA extraction method for deep subseafloor communities

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Many of the DNA-based researches have greatly enhanced our understanding on stratified nature in subseafloor microbial communities. An important prerequisite for DNA-based microbial community analysis is even and effective cell disruption for DNA extraction. With a commonly used DNA extraction kit, in average, roughly two-third of subseafloor sediment microbial cells remain intact (i.e., the cells are not disrupted), indicating that microbial community analyses may be biased at the DNA extraction step, prior to subsequent molecular analyses. To address this issue, standardized a new DNA extraction method using alkaline treatment and heating by precisely monitoring microbial cell numbers in the treated samples. Upon treatment with 1 M NaOH at 98oC for 20 min, over 98% of microbial cells in subseafloor sediment samples collected at different depths were disrupted. However, DNA integrity tests showed that such strong alkaline and heat treatment also cleaved DNA molecules into short fragments that could not be amplified by PCR. Subsequently, we optimized the alkaline and temperature conditions to minimize DNA fragmentation and retain high cell-disruption efficiency. The best conditions produced a cell disruption rate of 50-80% in subseafloor sediment samples from various depths, and retained sufficient DNA integrity for amplification of the complete 16S rRNA gene (i.e., ˜1,500 bp). The optimized method also yielded higher DNA concentrations in all tested samples compared with extractions using a conventional kit-based approach. Comparative molecular analysis using real-time PCR and pyrosequencing of bacterial and archaeal 16S rRNA genes showed that the new method produced an increase in archaeal DNA and its diversity, suggesting it provides better analytical coverage of subseafloor microbial communities than conventional methods.

Keywords: Subseafloor microbial community, DNA extraction, bias, archaea