

Preservation of ancient eukaryotic DNA in methane hydrate-associated marine sediments

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Ancient eukaryotic DNA in marine sediment can provide valuable information on the paleo-environment. Previously, ancient eukaryotic DNA has been retrieved from marine sediments associated with anoxic water column in lake and semi-closed sea, due to the minimized effect of oxic biodegradation [1, 2, 3, 4]. Cold seep sediment is characterized by high upward flux of methane, where there is the possibility that the excess energy source might suppress the degradation of deposited organic matter including nucleic acids by microbial activity. We investigated the preservation of ancient eukaryotic DNA in marine sediments associated with and without methane hydrate in the eastern Japan Sea, between which the dominant prokaryotic populations are clearly distinct [5]. Marine sediments were obtained by a giant piston corer during Marion Dufresne Cruise #179 in the eastern Japan Sea. For extraction of ancient eukaryotic DNA, two-step alkaline DNA extractions, conducted with 0.1 g of marine sediments, were newly developed [6]. DNA from living organisms such as fungi was removed by the first extraction under mildly heated conditions, which was followed by the second extraction. Eukaryotic DNA was successfully extracted and pyrosequenced from marine sediments associated with methane hydrate and ages up to ~90 ka. Based on 18S rRNA gene sequences, the diversity of ancient eukaryotic DNA was comparable between this study and previous ones. In addition, methane hydrate-associated sediments, which are globally distributed along the continental margin, have great potential to reconstruct the past terrestrial and marine ecology around the world over geological timescales.

[1] Coolen *et al* (2013) *PNAS* **110**, 8609-8614 [2] Boere *et al* (2011) *Organic Geochemistry* **42**, 1216-1225 [3] Edgcomb *et al* (2010) *EMI* **13**, 172-183. [4] Orsi *et al* (2013) *PLoS ONE* **8**, e56335 [5] Yanagawa *et al.* (2013) *JAES* (in press). [6] Kouduka *et al* (2011) *FEMS Microbiol Lett* **326**, 47-54