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A Method for Isolating High Purity RNA from Aqueous Samples for Radiocarbon Analysis

The deep terrestrial subsurface is inhabited by a diverse microbial population. The unique metabolic processes of subsurface microbial communities that allow them to survive in extreme environments may provide insight into the origins of life on earth and other planets. The deep gold mines of South Africa provide easy access to the deep terrestrial subsurface, as deep as 3500 meters, for collection of water, rock, and air samples for microbial and geochemical investigations. However, deep subsurface microbial processes remain a mystery largely due to the constraints in collecting and isolating uncontaminated samples. A variety of commercial kits and published protocols are available for the isolation, extraction, and purification of nucleic acids. However, it is difficult to extract and purify sufficient quantities (>100μg) of high purity RNA from aqueous samples for accurate radiocarbon (^{14}C) analysis because RNA is structurally less stable than DNA and rapidly degrades in the presence of the ubiquitous ribonuclease (RNase). The purpose of this study was to develop a method that yields high-purity RNA, free from non-RNA carbon contaminants, from laboratory Escherichia coli (*E.coli*) and aqueous samples collected from the Beatrix gold mine in South Africa for ^{14}C analysis. The ^{14}C analysis of microbial RNA from the Beatrix (BE) gold mine will help identify sources of organic carbon driving metabolic processes of the active microbial community. 194.3 μg of RNA with A260/280 of 1.95 and A260/230 of 2.51 and 675.5 μg of RNA with A260/280 of 2.05 and A260/230 of 2.39 was isolated from *E.coli* on grown acetate and LB media, respectively, for ^{14}C analysis. Additionally, 84.1 μg of RNA with A260/280 of 1.74 and A260/230 of 1.85 was isolated from BE326 samples for ^{14}C analysis.