obial Communities

RNA Preservation Solutio

C. K. Lee[1], S. J. Kennedy[2], A. Rueckert[1], V. Moroney[2], M. N. Brolaski[2],

[1]Thermophile Research Unit, University of Waikato, Hamilton, New Zealand; [2]MO BIO Labora
[3]College of Earth, Ocean, and Environment, University of Delaware, Lewes,

e Challenge

encing technologies for molecular microbial ore holistic views of microbial communities es in metabolic patterns and community lity to effectively capture "snapshots" of al to such studies. Given that on-location -freezing of samples are difficult to achieve nucleic acids for later extraction is often the /RNA and biostaticness are both critical for emperature conditions during transport and s on microbial communities [1]. Reagents have been known to be inadequate for soil tle or low quality RNA after extraction, thus ethods.

lution (Literally)

ovel reagent, the MO BIO LifeGuard™ Soil ity to stabilize total microbial RNA in soils temperatures. LifeGuard™ is a patented of nucleic acids through enzyme inhibition nal benefit of keeping samples biostatic [2].

al and Methods

ere used at a ratio (2.5X) of 5 ml of solution quality assessment) and various ratios for urdo Dry Valleys, Antarctica (for examining Soils were sieved and homogenized prior to were stored at indicated temperatures. RNA the MO BIO PowerSoil Total RNA Isolation mples were analyzed using agarose gel, Select samples were also analyzed using RT- RT-qPCR to evaluate extraction efficiency.

f Preserved RNA

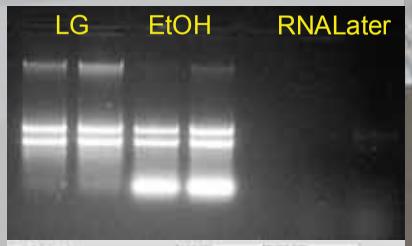
urity) of RNA extracted from soil samples EtOH, RNALater, and GITC/Solution D (5M r storage at 4°C and -20°C over a four-week high quality RNA at all time-points and

Figure 1

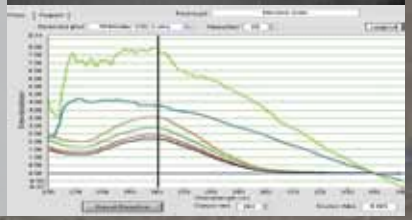
Figure 1A shows an agarose gel analysis of RNA extracted from soil samples preserved with LifeGuard™, 70% EtOH, and RNALater for two weeks at -20°C. 70% EtOH, while retaining some RNA, showed significant degradation; whereas nearly no RNA can be recovered from samples preserved with RNALater.

Figure 1B shows spectrophotometric (i.e. NanoDrop) profiles of RNA preparations from Figure 1A, and it is apparent that those of samples preserved in RNALater were highly abnormal. The degradation of RNA in 70% EtOH-preserved samples is not reflected in their NanoDrop profiles.

A.



B.



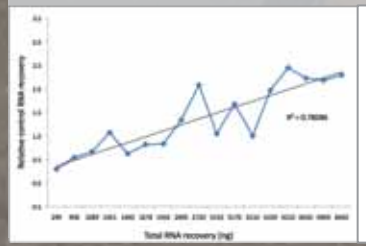
Quantification of Preserved RNA

Using armored RNA [3] as the internal standard for extraction efficiency, we found that the amounts of recoverable RNA declined with time and differed between various preservation solutions and temperatures. Both RNALater and GITC failed to yield any RNA beyond two weeks.

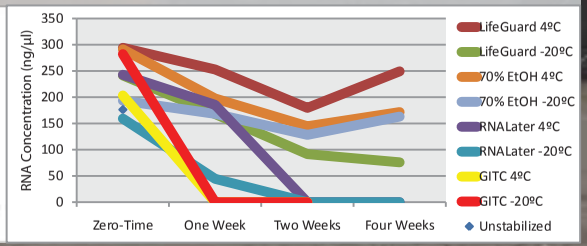
Figure 2

Figure 2A shows the relationship between total RNA and armored RNA recovery ratios. A linear correlation was found, suggesting the the amount of RNA extracted is indicative of recoverable RNA in the samples. Figure 2B shows the amounts of RNA recovered over the four-week period from various preservations solutions and temperatures.

A.



B.



Bacteriostatic Properties

To assess the conservation of microbial community structure in LifeGuard™-preserved samples, reverse transcription terminal restriction fragment polymorphism (RT-tRFLP) analysis using 16S rRNA gene-specific primers was performed on RNA samples extracted from preserved Dry Valley soils. The data indicated that after two weeks at -20°C, the RT-tRFLP profiles were statistically indistinguishable from those of the zero-time control samples. Storage at higher temperatures (i.e., 4°C) is possible for short periods, but LifeGuard™:Soil ratios lower than 2X should be avoided. Storage at warm temperatures (e.g., 37°C) potentially leads to inconsistency in observed microbial community structure.

Conclusi

Combining analyses of RNA quality and y RT-tRFLP, findings from our study sugge nucleic acid stabilizer and can effectively a under appropriate storage conditions. Life recovery of high quality total microbial R solutions, and is directly compatible with Isolation Kit. Additionally, because LifeGu bactericidal, it may also have applications i

Referenc

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2. J.P. Audia, M.C. Patton, and H.H. Winkler. Ap (24), 7809-7812
3. A. Rueckert and S.C. Cary. Limnol. Oceanogr.