Taking Snapshots of Microbial Communities

RNA-Based Assessment of Soil Microbial Communities Stored in a Novel Bacteriostatic RNA Preservation Solution

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The Challenge

The development of next-gen sequencing technologies for molecular microbial ecology research have allowed more holistic views of microbial communities and detection of minute changes in metabolic patterns and community structure. Undoubtedly, the ability to effectively capture "snapshots" of microbial communities is essential to such studies. Given that on-location DNA/RNA extraction and/or deep-freezing of samples are difficult to achieve for field research, preserving total nucleic acids for later extraction is often the only option. Stabilization of DNA/RNA and biostaticness are both critical for such preservation solutions since temperature conditions during transport and storage can have profound effects on microbial communities [1]. Reagents commonly used for such purposes have been known to be inadequate for soil samples in many cases, yielding little or low quality RNA after extraction, thus indicating a need for alternative methods.

The Solution (Literally)

We report the assessment of a novel reagent, the MO BIO LifeGuard™ Soil Preservation Solution, for its ability to stabilize total microbial RNA in soils stored up to 28 days at various temperatures. LifeGuard™ is a patented formula that protects the integrity of nucleic acids through enzyme inhibition (including RNase) with the additional benefit of keeping samples biostatic [2].

Material and Methods

LifeGuard™ and other solutions were used at a ratio (2.5X) of 5 ml of solution per 2 g of loam soil (for RNA quality assessment) and various ratios for mineral soils collected from McMurdo Dry Valleys, Antarctica (for examining extraction yield and biostaticness). Soils were sieved and homogenized prior to adding preservation solutions, and were stored at indicated temperatures. RNA extractions were performed using the MO BIO PowerSoil Total RNA Isolation Kit, and the extracted RNA samples were analyzed using agarose gel, NanoDrop, and RiboGreen assays. Select samples were also analyzed using RT-tRFLP to assess biostaticness and RT-qPCR to evaluate extraction efficiency.

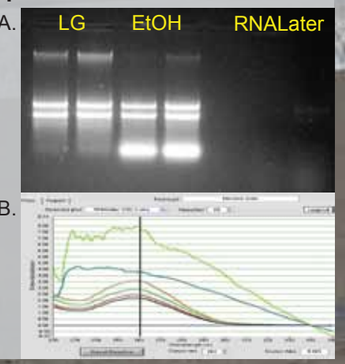
Quality of Preserved RNA

The quality (i.e., integrity and purity) of RNA extracted from soil samples preserved with LifeGuard™, 70% EtOH, RNALater, and GITC/Solution D (5M guanidine HCl) was examined after storage at 4°C and -20°C over a four-week period. Only LifeGuard™ yielded high quality RNA at all time-points and temperatures (Figures 1 and 2).

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.

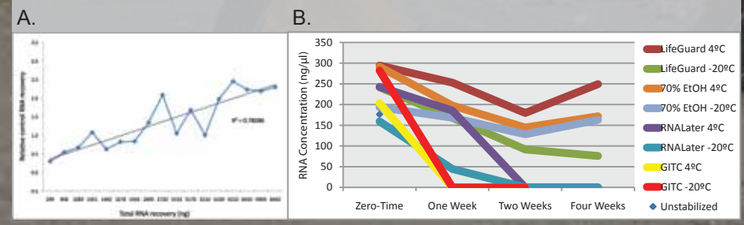


Quantification of Preserved RNA

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

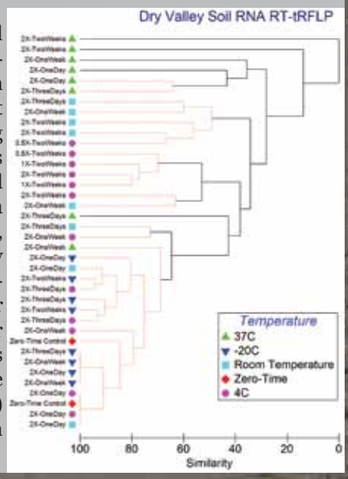
Figure 2A shows the relationship between total RNA and armored RNA recovery ratios. A linear correlation was found, suggesting the the amount of RNA extracted is indicative of recoverable RNA in the samples. Figure 2B shows the amounts of RNA recovered over the four-week period from various preservations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



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.

Figure 3



Conclusion

Combining analyses of RNA quality and yield, armored RNA RT-qPCR, and RT-tRFLP, findings from our study suggest that LifeGuard™ is an effective nucleic acid stabilizer and can effectively arrest microbial community changes under appropriate storage conditions. LifeGuard™ also offers more consistent recovery of high quality total microbial RNA than any other commonly used solutions, and is directly compatible with the MO BIO PowerSoil Total RNA Isolation Kit. Additionally, because LifeGuard™ is bacteriostatic rather than bactericidal, it may also have applications in cultivation-based studies.

References

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