Application Note

Microbial RNA protection in soil with Lifeguard™ Soil Preservation Solution

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Abstract

Analysis of soil metagenomics is ongoing by researchers worldwide looking at complex communities in field collected samples and extreme environments. The ability to preserve the microbial profile on site is not currently possible with existing reagents. Here we describe for the first time a new reagent, LifeGuard™ Soil Preservation Solution, which can preserve and minimize changes to the bacterial community within soils and maintain the nucleic acid integrity of samples for extraction after transport and storage. Community analysis using t-RFLP analysis and RNA integrity by agarose gel analysis confirmed that soil microbial communities are safely maintained in LifeGuard™.

Introduction

The ability to preserve the microbial community in soils during the collection process remains a problem for researchers studying ecological habitats in remote areas of the world. The microbiology of soil fluctuates in response to changes such as temperature, humidity, and light during the collection and transport process(1). Examination of the original profile of soil microbes allows for discovery of the actual species involved in maintaining the ecological balance of life under extreme conditions.

A major obstacle to preserving microbial communities in soil is the lack of a reagent that will keep microbial species intact while maintaining them in stasis over a range of temperature changes over time. A solution that is bacteriocidal will result in loss of information when the preservative is removed. However, a solution that is bacteriostatic allows for recovery of the original microcosm while preventing host enzymes from destroying nucleic acids in situ. The LifeGuard™ Soil Preservation Solution is the first reagent of its kind that can prevent microbial growth while maintaining nucleic acid integrity in samples stored up to 25^{o}C.

To examine the integrity of RNA extracted from soil preserved in LifeGuard™, samples of rose garden soil were stored in 2.5 volumes of solution per gram of soil and stored at temperatures of -20^{o}C, 4^{o}C, and room temperature for 30 days. For community analysis, soil collected from the Dry Valleys in Antarctica were stored in LifeGuard™ on site and compared to soils unstabilized and shipped to the lab at 4^{o}C. T-RFLP analysis was performed to look at the phylotypes recovered in preserved and unpreserved soil. All RNA was extracted using the RNAPowerSoil® Total RNA Isolation Kit.

Materials and Methods

Rose garden soil was used for time-course experiments. Per prep, 2 grams of soil was aliquoted into the Bead Tube supplied in the RNA PowerSoil® Kit. LifeGuard™ Soil Preservation Solution was added to the tube (5 ml) and the samples were mixed. Samples were stored at -20^{o}C, 4^{o}C, and at room temperature for 1 day, 1 week, and for 30 days. On the day of extraction, LifeGuard™ was removed by centrifugation of the bead tube at 2500 x g for 5 minutes. The supernatant was discarded, and the protocol for RNA PowerSoil® Total RNA Isolation Kit was followed as described. RNA samples were quantitated using the Nanodrop, and agarose gel electrophoresis was performed on 1% agarose gels with 10 ul of the final RNA sample.

For Antarctic dry valley soils, soil samples were collected in the field by Dr. Charles Lee from the University of Waikato, New Zealand, during the December 2008 field season and stored at 4^{o}C in LifeGuard™ Solution or the same soil was stored unstabilized for 30 days at 4^{o}C under simulated daylight. Temperature record placed within the samples indicated that significant temperature spikes were present during transit. Samples were stored in 2.5 ml of LifeGuard™ per 5 grams of Dry Valley soil. In separate experiments, soil was preserved in LifeGuard™ (0.5 ml per gram of soil) or in a diluted LifeGuard™ and then incubated 72 hours at room temperature. Samples were processed with the same RNA PowerSoil® Total RNA Kit, and cDNA was synthesized using a 16s rRNA primer and t-RFLP analysis performed.

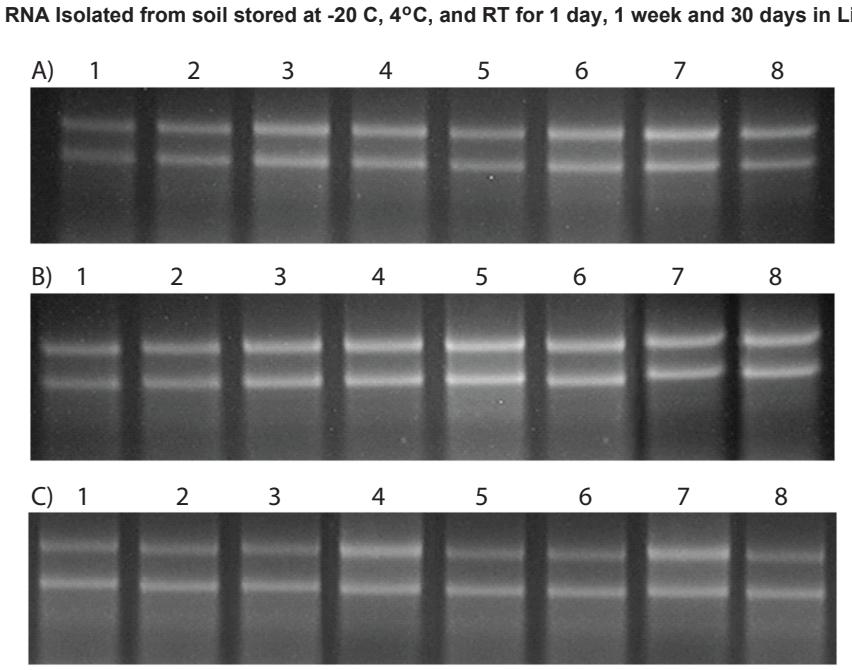

Figure 1 A-C. A) Soils stored for 1 day in LifeGuard™ B) Soil stored in LifeGuard™ for 1 week, and C) soil stored for 30 days in LifeGuard™. All samples were extracted with the RNA PowerSoil® Kit. Numbers across the top indicate the storage temperature as follows: 1+2= -20 °C, 3+4 = 4 °C, 5+6 = room temperature, 7+8 = fresh soil stored at 4 °C (control).

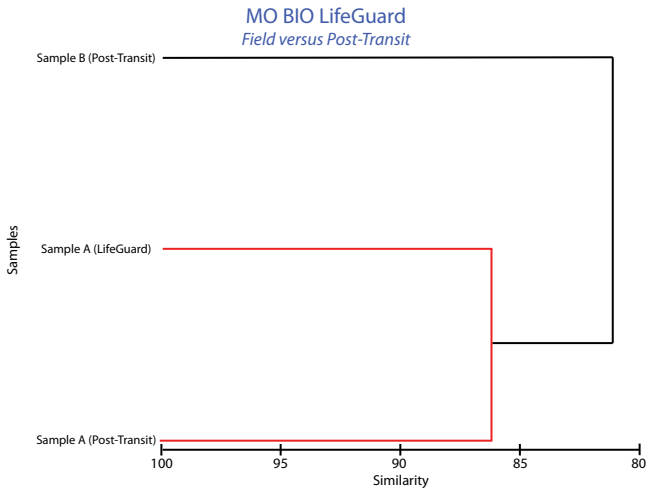

Figure 2. Antarctic Dry Valley soil samples were collected and immediately stabilized in LifeGuard™ Soil Preservation Solution and shipped at 4°C to New Zealand. Unstablized samples were shipped until identical conditions and stored for 30 days at 4°C under simulated daylight. Temperature record placed within the samples indicated that significant temperature spikes were present during transit. RNA was extracted from both stabilized and unstabilized samples using the RNA PowerSoil™ Total RNA Isolation Kit. Total full-length cDNA was generated from the RNA samples and analyzed using tRFLP to characterize bacterial communities present. Yields of RNA collected for the unstabilized soil (Sample A and Sample B, Post-Transit) were 7.4 ug and 4.8 ug, respectively, and 7.36 ug for stabilized soil (Sample A LifeGuard). tRFLP profiles of stabilized and unstablized Sample A are 86% similar, and additional phylotypes were detected in the unstabilized sample compared to the LifeGuard™ stabilized soil, indicative that community composition altered during transit and the time in storage. It is almost certain that the collective gene expression pattern of the community (i.e., metatranscriptome) has changed for the unstabilized sample and is significantly different to that observed in the field. (Data kindly provided by Dr. Charles Lee, University of Wakaito, NZ).

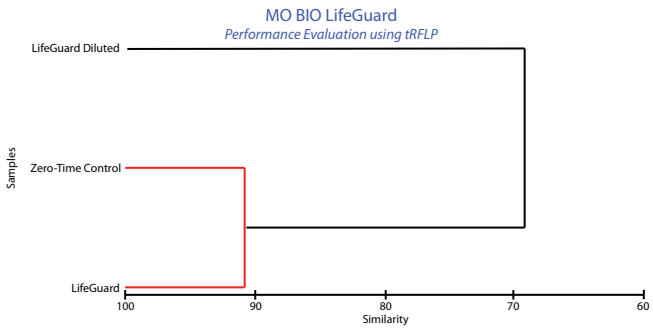

Figure 3. Antarctic Dry Valley soil was processed directly using the RNA PowerSoil™ Total RNA Isolation Kit after storage at 4°C, or preserved with LifeGuard™ Soil Preservation Solution, incubated for 72 hours at RT, then processed with the same kit. cDNA was generated from the RNA samples using a primer specific for bacterial 16S rRNA gene and analyzed using tRFLP to characterize bacterial communities present.
tRFLP profiles of stabilized and unstablized Sample A are over 90% similar, and the same sample preserved with a diluted LifeGuard solution has a significantly different tRFLP profile. The results indicating that soil preserved with LifeGuard™ yielded full length RNA with no observable loss in bacterial diversity compared to fresh samples. (Data kindly provided by Dr. Charles Lee, University of Wakaito, NZ).

To demonstrate the ability to recover intact RNA, a time-course experiment was undertaken that examined the integrity of RNA in samples stored at various temperatures. Soil stored in LifeGuard™ at -20^{O}C to room temperature for up to 30 days resulted in high yields of intact RNA (Figure 1A-C). Even though samples stored at -20^{O}C were frozen and then thawed, yields were not reduced and bacteria were protected during the thawing process. Additionally, we tested two freeze thaw cycles of soil in LifeGuard™ and we recovered intact and high yields of RNA (data not shown). Samples were stored at 37^{O}C for the length of the time-course, however, growth was not inhibited at this temperature, as expected for a bacteriostatic solution.

In collaboration with Dr. Charles Lee, microbial community analysis was performed with Antarctic Dry Valley soils collected on site that had been stabilized in LifeGuard™ and the same soil shipped unstabilized. Because of the low biomass needed to be preserved in the sample and lack of hydration in Dry Valley soils, a ratio of 0.5 ml LifeGuard™ per gram of soil was used. All samples were shipped at 4^{O}C and stored for 30 days. Total full length cDNA was generated from samples after RNA extraction and t-RFLP analysis performed. Results demonstrate that Sample A soils preserved and unpreserved were 86% similar in number of phylotypes but that the unpreserved sample had additional phylotypes, indicating that the fluctuations in temperature during transit resulted in changes to the original community (Figure 2). The collective gene expression pattern (metatranscriptome) of the community was altered during the time in storage. Sample B is a Dry Valley soil collected at a totally different site, and as expected, it gives a completely different transcriptional profile.

To determine the ability of the LifeGuard™ Solution to preserve the community gene expression profile at room temperature, a Dry Valley soil sample currently stored at 4^{O}C was mixed with LifeGuard™ and then stored at room temperature for 72 hours. The same soil was preserved with diluted LifeGuard™ to determine if the preservation is concentration dependent (Figure 3). The results show that the community was over 90% similar to the original soil and there was no observable loss in bacterial diversity compared to fresh samples. Diluted LifeGuard™ did not protect the gene expression profile confirming that profile did in fact change while sitting at room temperature for 3 days.

Discussion

A new reagent that preserves the microbial content of environmental samples and protects nucleic acids from degradation has been described. The LifeGuard™ Soil Preservation Solution is a unique formulation that protects bacterial diversity in samples over a range of temperatures over a span of 30 days (tested so far).

Previous work by our lab and others demonstrated the failure of other commercially available reagents (RNALater and RNA Protect) to preserve the bacteria in soils such that RNA could be extracted. The loss of information from precious and limiting soil samples collected at remote locations necessitated the need for a new solution for soil microbiologists. LifeGuard™ has the ability to protect nucleic acid integrity while keeping bacteria in stasis. Because samples stored at 37^{O}C did not remain in stasis, this demonstrates another important use for LifeGuard™. Microbes stored in LifeGuard ™ may also be recovered by culturing the bacteria out of the solution.

A number of different soils have been tested using LifeGuard™ and each soil will differ in its biomass and its saturation with water. Work performed on sediment samples indicated that a higher ratio of LifeGuard™ to sample may be required (3:1) due to dilution of the solution. Alternatively, room temperature storage of sediments in LifeGuard™ should be avoided if using a 2:1 ratio of LifeGuard™ to gram wet weight of sediment. Data on the results with sediment is pending t-RFLP analysis.

Finally, biomass content may play a role in the stabilization of soils at different temperatures and the ability of LifeGuard™ to freeze the community profile. We have shown that room temperature storage will be sufficient for a common "normal" soil at a 2:1 ratio of LifeGuard™ to soil while soils with low biomass and moisture were successfully stored with a 0.5:1 ratio. For a soil with unknown microbial content or where temperatures may exceed room temperature (22-25^{O}C), it may be preferable to store the soil at 4^{O}C to ensure the original community profile is maintained.

Analysis of microbial community gene expression in diverse locations rich in biodiversity is critical to our understanding of the balance of delicate ecosystems maintaining life all over the planet. LifeGuard™ Soil Preservation Solution at last provides a way to identify the true microbial interplay in irreplaceable samples and allows us to identify for the first time rare species of bacteria inhabiting the most remote places on earth.

References

1.. Saleema Saleh-Lakhaa, e, Michelle Millera, e, Rachel G. Campbellb, Kim Schneiderc, Parastu Elahimaneshd, Miranda M. Harta and Jack T. Trevorsa, (2005), Microbial gene expression in soil: methods, applications and challenges . Journal of Microbiological Methods, Volume 63, Issue 1, October 2005, Pages 1-19

Related Products and Ordering Information

Catalog No.	Description	Quantity
12868	LifeGuard™ Soil Preservation Solution	10 ml, 100 ml, 1 L, 7.5 L
12866-25	RNA PowerSoil® Total RNA Isolation Kit	25 preps
12867-25	RNA PowerSoil® DNA Elution Accessory Kit	25 preps
12888	PowerSoil® DNA Isolation Kit	50 preps, 100 preps
13111-V	Vortex Genie® 2 Vortex	1 unit
13000-V1	Vortex Adapters for Vortex Genie® 2	1 unit