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Instruments and Methods

Autonomous Microbial Sampler (AMS), a device for the uncontaminated collection of multiple microbial samples from submarine hydrothermal vents and other aquatic environments

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Abstract

An Autonomous Microbial Sampler (AMS) is described that will obtain uncontaminated and exogenous DNA-free microbial samples from most marine, freshwater and hydrothermal ecosystems. Sampling with the AMS may be conducted using manned submersibles, remotely operated vehicles (ROVs), autonomous underwater vehicles (AUVs), or when tethered to a hydrowire during hydrocast operations on research vessels. The modular device consists of a titanium nozzle for sampling in potentially hot environments ($>350\,^{\circ}C$) and fluid-handling components for the collection of six independent filtered or unfiltered samples. An onboard microcomputer permits sampling to be controlled by the investigator, by external devices (e.g., AUV computer), or by internal programming. Temperature, volume pumped and other parameters are recorded during sampling. Complete protection of samples from microbial contamination was observed in tests simulating deployment of the AMS in coastal seawater, where the sampling nozzle was exposed to seawater containing 1×10^6 cells ml^{-1} of a red pigmented tracer organism, *Serratia marinorubra*. Field testing of the AMS at a hydrothermal vent field was successfully undertaken in 2000. Results of DNA destruction studies have revealed that exposure of samples of the Eukaryote *Euglena* and the bacterium *S. marinorubra* to 0.5 N sulfuric acid at 23 °C for 1 h was sufficient to remove polymerase chain reaction (PCR) amplifiable DNA. Studies assessing the suitability of hydrogen peroxide as a sterilizing and DNA-destroying agent showed that 20% or 30% hydrogen peroxide sterilized samples of *Serratia* in 1 h and destroyed the DNA of *Serratia* in 3 h, but not 1 or 2 h. DNA AWAY™ killed *Serratia* and destroyed the DNA of both *Serratia* and the vent microbe (GB-D) of the genus *Pyrococcus* in 1 h.

Keywords: Microbiology; Samplers; DNA; Hydrothermal springs; Heat exchange; Aseptic

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1. Introduction

The study of the ecology, diversity and function of microbes in environments such as hydrothermal vents requires the marriage of culture-independent, molecular techniques of DNA sequencing and manipulation to determine the important members of the community and culture-dependent approaches to understand their physiology and functioning. In either case, it is essential that samples be obtained that are free from cross-contamination by microbes and microbial DNA from locations other than the site of sampling. Because most devices that are used to sample remote aquatic environments must first be transported through heavily contaminating waters (e.g., air–water interface near a ship, upper water column, etc.), means must be provided for protection of collected samples from such contamination or from cross-contamination between sampling sites. Since both viable microbes and/or DNA from the habitat of interest are analyzed, protection from both types of contamination is essential.

Obtaining contamination-free microbial samples from the marine and other remote environments has long been of interest (historical account in Zobell, 1941; see also Karl and Dore, 2001), beginning with hydrowire deployed samplers (e.g., Zobell, 1941; Niskin, 1962; Lewis et al., 1963) that implemented sterile evacuated bottles, bellows-like polyethylene bags or rubber bulbs to take samples. Proximity of the sample inlets to the hydrowire and initiation of sampling by unsterile components (e.g., smashing glass inlet tube by messenger or cutting inlet tubing with unsterile knife edge) lead to significant contamination issues, however. To tackle the contamination issue, Jannasch and Maddux (1967), developed a swing arm-like sampler that oriented into the current and mechanically drew samples into sterile syringes away from the hydrowire while a sterile dialysis bag was removed from the sterile inlet prior to sampling. Field tests, where the outer surfaces were purposely contaminated with a tracer organism, revealed dramatic improvements in the collection of uncontaminated samples (Jannasch and Maddux, 1967). However, the sampler was not widely implemented.

For sampling in deep waters where hydrostatic pressure is a parameter that can affect microbial viability and growth, pressure-retaining samplers have been implemented in a variety of designs for retrieval of samples in the absence of decompression. Examples include single sample versions with (Jannasch et al., 1973; Jannasch and Wirsen, 1977) and without (Tabor et al., 1981) sample inlet protection to reduce the potential for contamination. Bianchi et al. (1999) expanded the Jannasch et al. concept to allow up to eight pressurized samples to be taken during a single deployment, though inlet protection is less rigorous (alcohol-sterilized parafilm).

Microbial studies at hydrothermal vents have catalyzed development of an array of vent samplers that can obtain samples from hot environments. Malahoff et al. (2002), for example, have developed a sampling system that is able to take vent fluid samples and maintain both in situ temperature and pressure of the sample collected. Once aboard, ship subsamples can be transferred to multiple incubators without change in either pressure or temperature. Phillips et al. (2003) designed a sampler for the capture, temperature monitoring and in situ incubation of hot smoker fluids under vent conditions. In development at the Jet Propulsion Laboratory (http://www.jpl.nasa.gov/; A.L. Lane, L.C. French) is an Underwater Volcanic Vent Mission probe, an instrumented titanium probe that can be inserted into warm and hot water hydrothermal vents for in situ measurements of temperature, imaging and spectrographic analyses within the vent crevices for evidence of microbial growth in these high-pressure liquid environments.

Recent interests in sampling the Antarctic subglacial lake, Lake Vostok, for unique microbes that have been isolated from the surface biosphere for up to tens of millions of years (e.g., Siegert et al., 2003) have catalyzed the ongoing development of sampling devices that can aseptically penetrate the ice sheet to sample the subglacial water column and sediments, while maintaining the two biospheres isolated from one another (e.g., Blake and Price, 2002). Technology is also in development (e.g., Cryobot; Subsurface Ice Probe) to search for evidence of extraterrestrial life in the Martian icecaps (e.g., Cardell et al., 2004; Carsey et al., 2005) and possible sub-ice oceans on Europa (e.g., Carsey et al., 2000; French et al., 2001).

Most microbial samplers presently in use do not have sufficient inlet protection to guarantee freedom from exogenous contamination. To address this issue, we have developed an Autonomous Microbial Sampler (AMS) that obtains six microbial samples that are free from exogenous microorganisms or DNA. A technical description of the AMS and