In-Field Testing of Life Detection Instruments and Protocols in a Mars Analogue Arctic Environment A. Steele[1], M Schweizer[2], H.E.F. Amundsen[3] and N Wainwright[4] [1]Geophysical Laboratory, Carnegie Institution of Washington, Washington DC 20015, USA (a.steele@gl.ciw.edu), [2]Princeton University Department of Geology Princeton, USA., [3]Physics of Geological Processes, Univ. of Oslo, N-0316 Oslo, Norway, [4]Marine Biology Laboratory, WHOI, Woods Hole MA.

Introduction:

During August of 2003 an expedition including 19 scientists tested various equipment at a series of hot-springs sites on the island of Svalbard. This island is considered a Mars analogue environment due to the presence of hot springs, carbonate terraces and volcanic activity which have produced carbonate rosettes similar to those found in ALH84001. The goal was to test 4 portable instruments for their robustness as field instruments for life detection (for future human missions to Mars), to assess the Mars analogue environments for signs of life, to refine protocols for contamination reduction and to understand the effects of transport on sample integrity by assessing bioloads immediately in the field and then comparing this with laboratory measurements made after transportation.

Materials, Methods and Sites

The instruments used in this investigation were; *ATP luminometry* which assays for ATP as a measure of metabolic activity; *Scalar DG2 hand held digital microscope* which enables digital microscopy images to be captured at magnifications of up to x200. *Charles River Endosafe unit* which uses the Limulus Aemebocyte Lysate assay (LAL) and *Mobile PCR*, which is manufactured by MJ research and is a complete laboratory system containing thermocycler, transilluminator, gel imaging camera system, centrifuge and reagents. Ready mixed reagents (PCR beads) and extraction kits had been previously optimized to ensure contamination free extraction and field PCR. Novel DNA storage and extraction technology (Whatmans FTA paper) was used to supplement traditional techniques (MoBio soil extraction kit). Primer sets concentrated on the use of functional gene detection to characterize the metabolisms present at sites of geochemical interest. This was undertaken so as not to minutely characterize the speciation of the microbial population but to understand what microbial processes were taking place within the environment and to correlate this to geochemical measurements in real time.

The initial characterized site was Troll springs N-79^{o} 23.294, E – 13^{o} 26.392 156 ft elevation.. ATP and LAL analysis in-field were conducted. Samples were taken for extraction and subsequent PCR in the laboratory. Further visits to the site characterized the ATP activity in the dry terraces associated with the Troll springs site. Several sites were sampled including; Area 1. - Samples taken of the water course which contained green filaments. For this study these may be considered representative of the planktonic bacterial population of the main spring; Water temperature – 25.8^{o}C. Area 2. – Samples were taken of the mud at the bottom of the springs through which bubbles of gas could be seen emerging. White filaments of potential sulphur oxiding bacteria were seen to be part of the sample which may be considered as representative of the sessile community from the bottom of the pool. Water temperature – 26.4^{o}C. Area 3. – Moist sample of orange carbonate deposit at edge of pool. A black deposit beneath the sample was seen. Water temperature – 19.3^{o}C. Area 4. – Samples were taken at the base of an orange pool adjacent to main pool. This was a water and sediment sample through a black layer at the bottom of the pool. This sample may be representative of the microbial community at a point close to the main pool. Water temperature – 20.8^{o}C. Area 5. – A completely dry sample of broken carbonate next to area 4. Area 6. – Scrapings of the bottom of a pool furthest from source. This may be representative of the microbial population in pools far removed from the main pool. Water temperature – 11.1^{o}C. Area 7. 30M away from pool 1 and the main spring a small secondary spring. Within a small rivulet of the stream contained purple clumps of possible bacterial origin. Water temperature not recorded. Results of this analysis are shown in Table 1. Mcroscopy and ATP investigation of the dry terraces was undertaken after it was ascertained that under much of the outer layer of rock could be peeled away to reveal a green layer of probable cyanobacteria beneath. ATP analysis was conducted by swabbing a 5 x 5 cm area with an ATP free swab moistened by DEPC treated water. The end of the swab was then broken off and placed into a ethanol cleaned holder within the base plate of the instrument. An initial reading was taken without reagents to act as a control for background fluorescence and subtracted from a reading taken after addition of the reagents (as per manufacturers instructions). Triplicate measurements were taken of areas on top of the terrace structure shown in Figure 1 and at the base of the structure. Significance testing was performed using a twin tailed T-test (P=0.05). Microscopy was preformed using the DG2 digital microscope at x30, x100 and x200.

Results

Eubacterial DNA was extracted and amplified for all samples. Degenerate eubacterial primers showed a

weak PCR band of the correct size in the extraction blank. During extraction the integrity of the glove bag was compromised and so contamination of the extractants cannot be ruled out. Due to the probable derivation of these contaminants as from within the laboratory and that extraction blanks remained blank for all other PCR reactions we conclude that contamination does not affect the results from the other PCR runs as the genes targeted by functional gene analysis are unlikely to be present in lab contaminants which was confirmed by negative extraction blanks. Sequencing of the eubacterial primers including the blank should allow any contaminants to be identified and discounted from each area. In field PCR at another site (Trollolsen) showed a negative exctraction blank and the presence of sulphur oxidizing species (results not shown but will be presented).

Table 1 PCR results from Troll springs

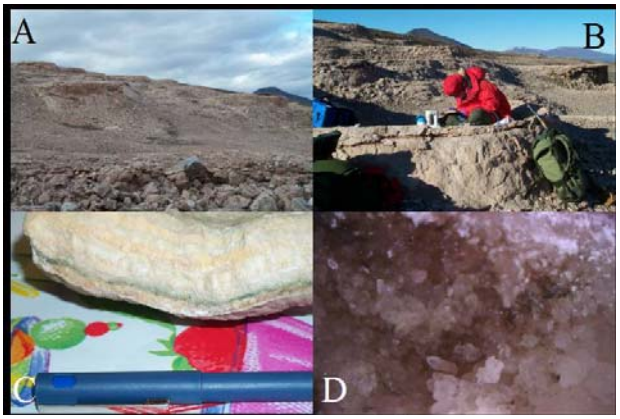
Primer / Area	Eub	Pb	GS	GNS	HB	Nr	Ao	APS	Mcr A
1	+++	+	+	-	++	+	-	++	-
2	+++	+++	+++	+	-	+	-	+++	+++
3	+++	+++	-	-	-	-	-	++	-
4	+++	+++	-	-	-	-	-	-	-
5	+++	+++	-	-	-	-	-	-	-
7	+++	+++	-	-	-	-	-	+++	-
Blank	+	-	-	-	-	-	-	-	-
PCR blank	-	-	-	-	-	-	-	-	-

Eub – Eubacterial; Pb – Purple Bacteria; GS – Green sulphur bacteria; GNS – Green Non sulphur bacteria; HB – Heliobacteria; Nr – Nitrate reductase; Ao – Amonia oxidase; APS – APS reductase; McrA – Methanogenesis. - - negative; + - weakly positive, ++ - medium positive (similar band fluorescence to ladder) +++ - strongly positive.

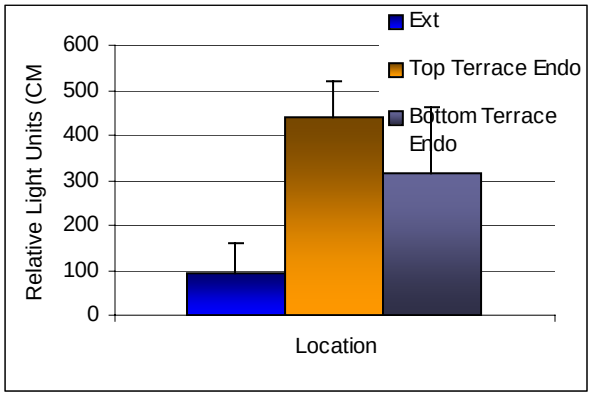
The distribution of anaerobic photosynthetic bacteria, sulphur oxidizing bacteria, methanogens and nitrate reducing bacteria are currently being overlayed on detailed 3-D maps of the site and will be presented. Furthermore the results of ATP and LAL analysis

A large extent of the terraces shown in Figure 1a show the presence of endolithic growth. There is significant increase in the recorded metabolic activity from the outside surface of the carbonate compared to the cryptoendolithic layer both in the upper and lower terraces (graph 1). In many places this layer exists as a dense green covering on the bottom of a crust of carbonate easily removed from the host rock. There is a small but statistically insignificant drop in the amount of metabolic activity in the top terrace compared to the base. Interesting the metabolic activity appears to be higher in the endolithic communities than in the pools above (with the possible exception of the dense biofilm in the source pool which was not measured).

Figure 1.A – Is an image of the relic terraces. B – Is a picture of the ATP analysis being undertaken on the lower terrace by M Schweizer C – Is an image of a sample of the terrace showing the layering the carbonate and the presence of a layer of cyanobacteria typical of a cryptoendolithic community. D – Is a x30 magnification digital image showing the presence of dark green cyanobacterial colonies through the matrix of the sample shown in C.



Graph 1 shows the ATP luminometry data for Dry terraces. (Ext – External surfaces of both top and bottom terraces).



Conclusions

We were able to detect and quantify bacterial load from several sites including enolithic communities from carbonate dry terraces at the site (Figure 1). We have been able to use functional gene analysis in the field to rapidly constrain the types of bacterial activity being undertaken within the hotsprings site. This is the first time that these types of examinations were carried out in the arctic.

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