SPECTRAL CHARACTERIZATION OF HALOPHILES ISOLATED FROM HYPERSALINE ENVIRONMENTS RELEVANT TO MARS AND OCEAN WORLDS. G. J. Garcia[1], E. C. Sklute[2], M. A. Schneegurt[3], J. F. Holden[1], and M. D. Dyar[2,4], [1]Dept. of Microbiology, Univ. of Massachusetts, 639 North Pleasant Street, Amherst, MA 01003, gjgarcia@umass.edu, [2]Planetary Science Institute, 1700 East Fort Lowell, Tucson, AZ 85719, [3]Dept. of Biological Sciences, Wichita State Univ., 1845 Fairmount St., Wichita, KS 67260, [4]Dept. of Astronomy, Mount Holyoke College, 50 College St., South Hadley, MA. 01075.

Introduction: A goal of exobiology is to use spacecraft-relevant instrumentation to identify and characterize signatures of microorganisms from terrestrial environments that are analogous to what may be found on other planets [1]. To prepare for current and future planetary science missions, *in situ* microbial biosignature detection is sought using spectroscopic techniques. To this end, reflectance spectra of four highly pigmented halotolerant bacteria were examined to determine if spectral differences between these strains can be identified, and to search for spectrally diagnostic biomolecules. *Halomonas* sp. GSP3, *Halomonas* sp. BLE7, *Nesterenkonia* sp. HL64 and *Marinococcus* sp. HL11 were isolated from the following Mars-analog hypersaline environments: Great Salt Plains (GSP3) (saturated with NaCl), Basque (BLE7), and Hot Lake (HL64 & HL11) (saturated with $MgSO_4$). It is likely that permafrost would contribute to the presence of heavy brines dominated by various salt species, including $MgSO_4$, $FeSO_4$, and NaCl compositions that may also be relevant on ocean worlds [2].

Fourier transform infrared attenuated total reflectance (FTIR-ATR) and visible-to-near infrared (VNIR) spectroscopies were used to characterize the biological samples. Using a multi-instrument approach, selection rules and detection limits of each instrument were addressed [3]. Insights were gained into potential biosignatures found in terrestrial and extraterrestrial samples and biosignature interpretation.

Methods: Bacterial isolates were cultured on modified growth media with varying salinities [4-6]. Cell biomass was concentrated via centrifugation at 10,000 × g at 4 °C for 10 min, followed by two washes using deionized H_2O. Following a drying period, cell material was broken into powder-like fragments for FTIR analysis. For VNIR analysis, cell material was placed on a matte black sample cup and dried in place. Both bulk and micro-Raman spectroscopies were attempted on all samples but extreme fluorescence for all samples except *Nesterenkonia* sp. HL64 prevented interpretation.

FTIR spectra were acquired using a Bruker Vertex 70 FTIR spectrometer with diamond ATR attachment and a wide-angle beam splitter and detector (30-6000 cm^{-1}, 4 cm^{-1} spectral resolution, Mertz phase correction, Norton-Beer medium apodization, 128 scans/sample). Continuum removal was performed using Bruker's OPUS software with a concave rubber band correction. VNIR spectra were collected using an ASD Fieldspec4 Max spectrometer with incidence and emission angles set to 30° and 0°, respectively. Continuum removal was performed on DEVAS using the convex hull "rubber band" baseline removal algorithm.

Results: Biomass analyzed using FTIR resulted in spectra with characteristic absorption bands for a combination of biomolecules found in the sample (i.e., lipids, proteins, carbohydrates, nucleic acids). The main differences between the samples were found in the lipid and protein regions of the spectra (Fig. 1). The broad band at 3280 cm^{-1} is consistent with Amide A (assigned to the N-H stretching vibration) overlain on a broad hydration feature. Absorption bands corresponding to lipids occur in several regions where the region between 2950-2850 cm^{-1} is assigned to the symmetric and asymmetric stretching vibrations of $-CH_2$ and $-CH_3$. The highest and lowest peak intensities detected within this region were for *Halomonas* sp. BLE7 and *Marinococcus* sp. HL11, respectively, at 2923 cm^{-1}. The lipid region between 1450-1400 cm^{-1} showed differences among band intensities for all the species and includes a prominent shoulder at 1378 cm^{-1} for *Nesterenkonia* sp. HL64.

The Amide I and II bands (1700-1500 cm^{-1}) are determined by the stretching vibrations of the C=O and C-N groups (Amide I), and the N-H (Amide II) group. A plateau between the Amide peaks is apparent in the *Marinococcus* sp. HL11 spectrum, likely due to the absence of the shoulder seen in the other spectra at ~1600 cm^{-1}. Another shoulder was detected in this region at 1646 cm^{-1} for *Halomonas* sp. BLE7 that was not seen in spectra for the other species. Differences were found between microbial species in the Amide III bands (1350-1200 cm^{-1}), where small shifts in relative band intensities and locations were observable. For example, *Halomonas* sp. BLE7 exhibits a shoulder present at ~1337 cm^{-1} not detected among the other spectra.

The spectral region between 1242-972 cm^{-1} is associated with vibrations of phospholipids and the sugar-phosphate backbone [7]. Absorptions near 1234 cm^{-1} occurred at varying relative intensities across species, with *Halomonas* sp. BLE7 displaying the strongest absorption (with respect to other peaks in the spectrum). In addition, the band envelope centered near ~1150 cm^{-1} was similar for *Nesterenkonia* sp. HL64 and *Marinococcus* sp. HL11 and between *Nesterenkonia* sp. HL64

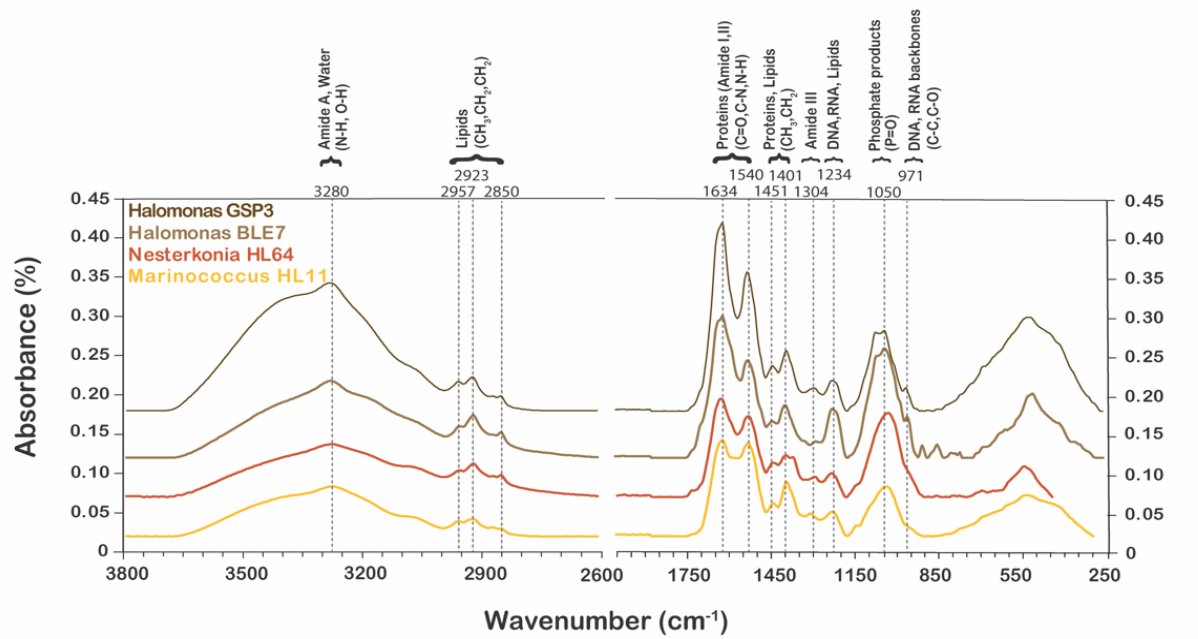

Figure 1. Relative intensity of Fourier transform infrared (FTIR) spectra of *Halomonas* sp. GSP3, *Halomonas* sp. BLE7, *Nesterenkonia* sp. HL64, and *Marinococcus* sp. HL11.

and *Marinococcus* sp. HL11, but distinct between the two groups of microbes. This difference was apparent in prominent bands at 1050 and 1080 cm^{-1} in the two *Halomonas* sp. spectra that are not found in the spectra of the other two organisms. Spectra for *Halomonas* sp. BLE7 showed considerable differences between all other organisms in the region between 930-765 cm^{-1}; additional research is required to identify these bands.

VNIR spectra for the salinotolerant bacteria are color-coded to reflect the pigment color of sampled biomass (Fig. 2). Distinct reflectance increases in the VIS to NIR region are observed between 0.68 and 0.72 μm

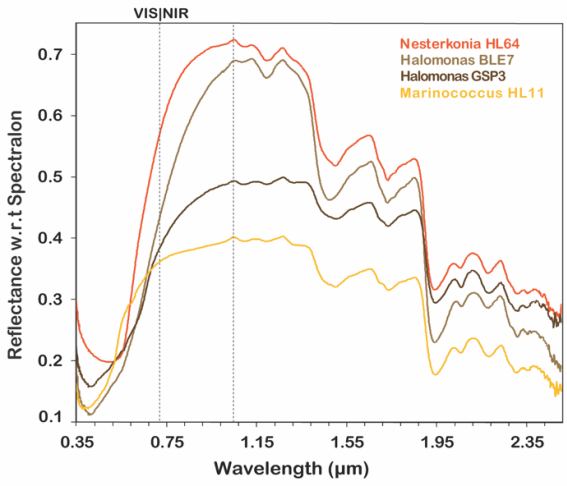

Figure 2. VNIR spectra of *Nesterenkonia* sp. HL64, *Halomonas* sp. BLE7, *Halomonas* sp. GSP3, and *Marinococcus* sp. HL11.

and reflect the color of the biomass. *Nesterenkonia* sp. HL64 (red) exhibited the largest reflectance increase, followed by *Marinococcus* sp. HL11 (yellow/tan), *Halomonas* sp. BLE7 (yellow/brown), and *Halomonas* sp. GSP3 (brown). All organisms exhibit reflectance maxima at ~1.00 μm that may be indicative of the bacteriochlorophyll *b*, as described in [9]. A distinctive absorption at 0.56 μm is seen for *Halomonas* sp. GSP3 and is consistent with an absorption in the protein pigment phycoerythrin [10]. Similar, strong absorptions are observed >1.55 μm, likely due to biomass pigments [8] and hydration features. Subtle differences between 1.0 and 1.55 μm should be investigated further for species differentiation.

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