EXTREMELY LOW LEVELS OF DISPERSAL OBSERVED FOR HUMAN-ASSOCIATED MICROBES INTO A LOCAL PRISTINE TERRAIN DURING A SIMULATED MOON TRAVERSE ON SEA ICE ALONG THE NORTHWEST PASSAGE IN THE ARCTIC.

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Introduction: Future human missions to the Moon and Mars will transport terrestrial microorganisms to the sites of exploration. This will be an unintended experiment in directed panspermia [1]. Although the Moon is considered to be free of conditions that would easily support microbial activity, and is likely sterile, the Moon can be used for testing hardware, operations, and protocols slated for use during Mars explorations. The objective of the current study was to determine if aerial dispersal of human-associated microorganisms occurred during a simulated rover traverse over snow.

Methods: During the week of April 10-17, 2009, the Mars Institute's *Moon-1 Humvee Rover* (Fig. 1) was driven a distance of 496 km on sea-ice along the Northwest Passage, from Kugluktuk to Cambridge Bay, Nunavut, Arctic Canada. This *Northwest Passage Drive Expedition* was carried out under the auspices of the NASA Haughton-Mars Project in support of field studies of pressurized rovers in future Moon/Mars human exploration. The *Moon-1* rover (a modified military ambulance Humvee) is a highly capable non-pressurized all-terrain rover simulating some of the basic attributes of a pressurized planetary rover, including the ability to traverse unprepared terrain and offer shelter to a crew of five. The diesel-powered *Moon-1* was accompanied by two gasoline-powered snowmobiles, each vehicle pulling a *komatik* sled loaded with food, fuel, and other equipment and supplies. Among the science experiments conducted during the traverse was a planetary protection investigation designed to measure the amount of human associated microbes released into a "pristine" environment as a result of exploring it with a large crewed rover.

Samples of surface snow and grit were collected in sterile 50 cc plastic tubes at three sites along the traverse: Sites A, B, and C (Table 1). At each of these sites, six tubes of samples were collected systematically in specific locations in relation to the Humvee: 1 = inside, on driver side floor; 2 = inside, in drainage gutter on rear access steps; 3 = outside, upwind; 4 = outside, downwind; 5 = outside, up track; 6 = outside, down track (Fig. 2). Site A was a brief science stop during Day 1 of the 7-day traverse. Sites B and C were overnight sites with samples collected in the morning. Food was prepared and consumed outside the Humvee within 5 m of the rear of the vehicle at Sites B and C.

Fig. 1: *Moon-1 Humvee Rover* being offloaded in Kugluktuk, Canada.

Fig. 2: *Moon-1* sampling pattern for human associated microorganisms.

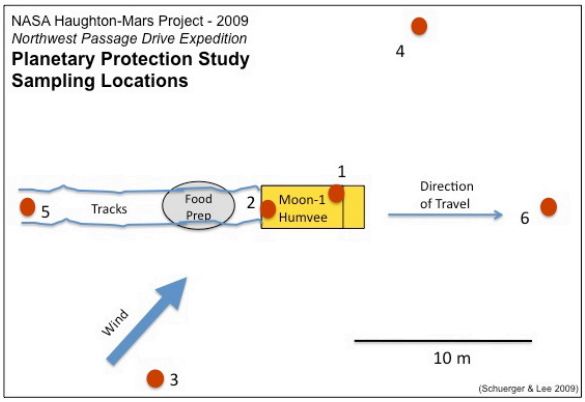

Table 1: *Moon-1* traverse summary data.

Site	Coordinates	Temp	Wind	Weather
A	67°49.97'N, 115°01.19'W	-2.2°C	SW 20 kts	Overnight Snow
B	67°44.12'N, 113°54.33'W	-6.8°C	SW Calm	Overnight SW winds
C	69°01.02'N, 105°50.06'W	- 5°C	SW 20 kts	Overnight Snow

Microbial characterization. Snow and ice samples collected during the *Moon-1* traverse were kept frozen (-25 to -5 C) during the traverse, and then shipped on ice to microbiological labs at Kennedy Space Center, FL for processing (Schuerger lab). Aliquots of all

snow samples were plated on R2A agar plates at the undiluted rates obtained after melting the snow/ice samples at 4 C for 48 hrs. Sets of petri plates from all samples were incubated at 37, 24, or 4 C, and the numbers of colony-forming units (cfu's) per sample and temperature were estimated after 7 and 28 days of incubation. Unique colonial morphotypes were recovered and purified from all petri plates; over 200 individual isolates of eubacteria and fungi were collected and archived.

All recovered isolates of eubacteria and fungi were processed with 16S and 18S sequencing, respectively. Eubacterial 16S primers B27F (5'-GAGTTTGATC MTGGCTCAG-3') and B1512R (5'-AAGGAGGTGA TCCANCCRCA-3') were used as described previously [2]. Eukaryotic 18S rDNA sequences were amplified using the universal eukaryotic primers 5'-GGAGGGCAAGTCTGGT-3' and 5'-ACGGGCG GTGTGTRC-3' [3,4]. DNA samples were amplified by polymerase chain reaction (PCR) [5]. 16S and 18S sequences were then completed by the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida.

Results: All samples collected from within the *Moon-1* rover were heavily populated by both eubacterial and fungal species. Population densities taken from samples within the *Moon-1* rover ranged between 9 and 150 cfu/ml for cultures incubated 7 d at 37 C, and from 10 to 1.1 x 10^3 cfu/ml for cultures incubated 28 d at 24 and 4 C. All snow samples from the upwind, downwind, uptrack, and down track sample sites exterior to the *Moon-1* rover were negative for both eubacteria and fungi except for two cfu's recovered at Site A from sample A4 (downwind) and A5 (uptrack). Thus, while the internal samples from the *Moon-1* rover contained a wide range of colony morphotypes of eubacteria and fungi, only two individual colonies of eubacteria were recovered from any samples of snow collected from the pristine local terrain around the rover. The two isolates of eubacteria were identified through 16S sequencing as *Breivibacillus agri* (NCBI acsension #AJ586380; 0.975 closest match). Furthermore, the recovery of two isolates of this bacterium occurred on two petri plates incubated at 24 C. No eubacteria or fungi were recovered from any of the snow samples (i. e., from all three sample sites) incubated at 4 or 37 C.

All isolates recovered will be identified through 16S or 18S sequencing using the BLAST library on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). As of this writing, approximately 30% of the isolates recovered from all *Moon-1* or snow samples have been sequenced and identified. The dominant species recovered from within the *Moon-1* rover are *Bacillus circulans, B. licheniformis, B. megaterium, B. psychrodurans, B. subtilis, B. simplex, Brevibacillus borstelensis, Kocuria rosea, Microbacterium paraoxydans, Paenibacillus pabuli, P. illinosensis, P. amlolyticus*, and S*porosarcina aquimarina* (based on NCBI closest match values > 0.975).

Discussion: The overall results support the conclusion that common human-associated microorganisms were not dispersed easily onto the terrain surrounding the *Moon-1* rover. The sampling was designed to investigate contamination of virgin snow aound the *Moon-1* vehicle as a prediction of shedding from the rover and humans into the air column in which dispersal was via air currents. Because of the recovery of only two colonies on hundreds of R2A petri plates, it is plausible that these could be contaminants emplaced into the snow samples during the collection process in the field or as lab contaminants during processing. But clearly, contamination of the three sample sites along the 496 km traverse of the *Moon-1* rover did not occur at a high level.

Future studies should include sampling the areas immediately adjacent to simulated rovers where humans walk. It is likely that where the human operators actually stepped and worked were contaminated at higher levels than identified here. But again, our objective was to monitor aerial dispersal of human-associated microorganisms to pristine locations around the rover. The implications for a life-detection mission to Mars is that even if human crew members are involved in collecting field samples, as long as they are using sterilized implements when interacting with the terrain, they are likely not to contaminate the sample sites. Furthermore, two additional factors will be present on Mars that will reduce the chances of contaminating regolith or rock samples during human missions: (1) all humans will be fully contained within spacesuits, which will greatly isolate their indigenous microflora during the EVA activities, and (2) the surface of Mars has at least 13 biocidal factors [6] that will increase the inactivation of human-associated microbes on the outsides of space suits, thus reducing viable bioloads on equipment and spacesuits. Thus, it is likely that the biocidal stresses on Mars will be several orders of magnitude "harsher" than were encountered during the *Moon-1* mission.

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