Transcriptomic responses of *Serratia liquefaciens* cells grown under simulated Martian conditions of low temperature, low pressure, and CO$_2$-enriched anoxic atmosphere

Patricia Fajardo-Cavazos$^1$, Michael D. Morrison$^1$, Kathleen M. Miller$^1$, Andrew C. Schuerger$^2$ & Wayne L. Nicholson$^1$

Results from previous experiments indicated that the Gram-negative α-proteobacterium *Serratia liquefaciens* strain ATCC 27592 was capable of growth under low temperature (0 °C), low pressure (0.7 kPa), and anoxic, CO$_2$-dominated atmosphere—conditions intended to simulate the near-subsurface environment of Mars. To probe the response of its transcriptome to this extreme environment, *S. liquefaciens* ATCC 27592 was cultivated under 4 different environmental simulations: 0 °C, 0.7 kPa, CO$_2$ atmosphere (Condition A); 0 °C, ~101.3 kPa, CO$_2$ atmosphere (Condition B); 0 °C, ~101.3 kPa, ambient N$_2$/O$_2$ atmosphere (Condition C); and 30 °C, ~101.3 kPa, N$_2$/O$_2$ atmosphere (Condition D; ambient laboratory conditions). RNA-seq was performed on ribosomal RNA-depleted total RNA isolated from triplicate cultures grown under Conditions A-D and the datasets generated were subjected to transcriptome analyses. The data from Conditions A, B, or C were compared to laboratory Condition D. Significantly differentially expressed transcripts were identified belonging to a number of KEGG pathway categories. Up-regulated genes under all Conditions A, B, and C included those encoding transporters (ABC and PTS transporters); genes involved in translation (ribosomes and their biogenesis, biosynthesis of both tRNAs and aminoacyl-tRNAs); DNA repair and recombination; and non-coding RNAs. Genes down-regulated under all Conditions A, B, and C included: transporters (mostly ABC transporters); flagellar and motility proteins; genes involved in phenylalanine metabolism; transcription factors; and two-component systems. The results are discussed in the context of Mars astrobiology and planetary protection.

A central goal of Astrobiology is to understand the potential for habitability in the universe, including determination of the physical limits at which life can exist and the mechanisms used by living organisms to survive and grow in extreme environments$^{1,2}$. Of particular interest have been investigations of whether Earth life could inhabit the environments of Mars, our closest potentially habitable neighbor. These studies are relevant to two related areas: (i) the potential for transport of life between Earth and Mars by natural impact processes (i.e., lithopanspermia), and (ii) the potential forward contamination of Mars as a consequence of human exploration activities (i.e., planetary protection)$^{3-7}$. Due to their well-known resistance properties and ubiquitous distribution in extreme terrestrial environments, prokaryotes are considered the most likely candidates for interplanetary transfer by natural processes or human spaceflight activities, and much attention has logically focused on highly resistant or extremophilic microbes such as spores of *Bacillus subtilis* or the extremely radiation-resistant *Deinococcus*...
Streptomyces genera permafrost, and they were identified by 16S rDNA sequencing as hardy environmental species belonging to the icy conditions in the near subsurface of Mars. The origin of these bacteria were mostly Arctic soils or Siberian culture (0 °C), low pressure (0.7 kPa) and atmospheric gas composition (anoxic CO2) intended to simulate the physiological versatility of bacterial species have been discovered that can grow under simultaneously applied conditions of low temperature, applied either singly or in pairwise combination. Although most bacteria were unable to grow under the conditions applied in these early experiments, recently a small but growing subset of bacterial species have been discovered that can grow under simultaneously applied conditions of low temperature (0 °C), low pressure (0.7 kPa) and atmospheric gas composition (anoxic CO2) intended to simulate the physical conditions in the near subsurface of Mars. The origin of these bacteria were mostly Arctic soils or Siberian permafrost, and they were identified by 16S rDNA sequencing as hardy environmental species belonging to the genera Bacillus, Carnobacterium, Clostridium, Cryobacterium, Exiguobacterium, Paenibacillus, Rhodococcus, Streptomyces, and Trichococcus.

Early studies confirmed that the UV radiation environment was a potent factor limiting the survival of Earth microbes on the martian surface, but that burial of cells at even minimal depths in the regolith provided effective UV shielding. Subsequent studies testing the ability of various bacteria to grow under increasingly Mars-like laboratory simulations revealed that growth of most of the microbes tested, mostly strains obtained from laboratory collections or spacecraft assembly facilities, was inhibited by low temperature, low pressure, and anoxia, predominantly CO2 atmospheres, applied either singly or in pairwise combination. Although most bacteria were unable to grow under the conditions applied in these early experiments, recently a small but growing subset of bacterial species have been discovered that can grow under simultaneously applied conditions of low temperature (0 °C), low pressure (0.7 kPa) and atmospheric gas composition (anoxic CO2) intended to simulate the physical conditions in the near subsurface of Mars. The origin of these bacteria were mostly Arctic soils or Siberian permafrost, and they were identified by 16S rDNA sequencing as hardy environmental species belonging to the genera Bacillus, Carnobacterium, Clostridium, Cryobacterium, Exiguobacterium, Paenibacillus, Rhodococcus, Streptomyces, and Trichococcus.

Recently a screen of laboratory strains of bacteria for growth under the above “Mars-like” environmental conditions resulted in the identification of several species of the genus Serratia able to grow, including the type strain of S. liquefaciens, strain ATCC 27592. Although in early publications the interest in S. liquefaciens stemmed from its being an opportunistic human pathogen, recent reports point towards its physiological versatility allowing it to occupy ecologically diverse environments such as cold raw milk, thawed cryoprecipitate23 or pulp mill effluent24. Because Serratia spp. have also been found on and within spacecraft and their assembly facilities26,27, S. liquefaciens is considered a potential forward contaminant of Mars-bound missions. The discovery that S. liquefaciens is capable of growth under Mars-like environmental conditions naturally leads to the question: what are the cellular and molecular mechanisms responsible? As a first step towards addressing this question, here we investigated how the global transcription profile (i.e., the transcriptome) of this organism responds when cultivated under environmental conditions mimicking those found in the martian near-subsurface. This study represents the first transcriptome profiling of a microorganism exposed to a simulation of the physical conditions prevailing on Mars.

Results and Discussion
Characterization of the S. liquefaciens transcriptomic response to different physical environments. RNA-seq was utilized to analyze transcriptional changes in S. liquefaciens strain ATCC 27592 in response to four environmental conditions of temperature, pressure, and atmospheric gas composition simulating the physical environment of Mars (Condition A), Earth-ambient laboratory conditions (Condition D), or a mixture of the two environments (Conditions B and C) (Table 1). Total RNA was isolated from cells grown under each condition as described in Materials and Methods, and determination of RNA integrity number (RIN) values (Table 1) demonstrated that all RNA samples were of the high quality required for further processing. RNA-seq analysis was performed on three replicates from each condition, resulting in 12 libraries which were sequenced on an Illumina HiSeq2500 instrument and subjected to the bioinformatic and statistical workflow described in detail in Materials and Methods and summarized in Fig. 1. Transcripts were defined as significantly differentially expressed if they exhibited a >4-fold difference with an adjusted P value < 0.01.

Overview of transcriptome analysis. In order to elucidate differences in gene expression of S. liquefaciens under the four conditions tested, each of the datasets obtained from Conditions A, B, or C were subjected to pairwise comparison with dataset D serving as the “Earth-like” control. The comparative analysis identified 493 differentially expressed genes in Condition A (193 up- and 300 down-regulated); 708 genes in condition B (209 up- and 499 down-regulated) and 429 genes in Condition C (153 up- and 276 down-regulated) with respect to the reference Condition D. The results of this analysis are summarized in Table 2 and represented graphically as Venn diagrams (Fig. 2). Inspection of the data revealed that exposure of S. liquefaciens to Conditions A, B, or C each produced its own set of transcriptome responses. These sets were partially unique and partially overlapping in any combination examined (Fig. 2). For example, a common set of 34 up-regulated and 156

| Condition | A   | B    | C    | D    |
|-----------|-----|------|------|------|
| Temperature (°C) | 0   | 0    | 0    | 30   |
| Pressure (kPa) | 0.7 | −101.3 | −101.3 | −101.3 |
| Gas Composition | CO2, anoxic | CO2, anoxic | 80% N2/20% O2 | 80% N2/20% O2 |
| Incubation time (days) | 7   | 23   | 7    | 1    |
| Number of TSA plates harvested per replicate | 1   | 8    | 1    | 1    |
| RIN values of replicates | 9.6, 8.8, 8.8 | 9.4, 9.6, 8.9 | 9.5, 8.8, 9.3 | 9.0, 9.5, 9.2 |

Table 1. Environmental conditions used in this study.
down-regulated transcripts were found in cells exposed to all three Conditions A, B, and C; on the other hand, exposure to Condition A produced differential expression of 112 genes unique to Condition A (75 up- and 37 down-regulated) (Fig. 2). An Excel file listing the differential transcripts in common with Conditions A, B, and C vs. D is presented in Supplemental Table S1.

Expression differences due to temperature. The transcriptome of *S. liquefaciens* ATCC 27592 consists of 5,003 total genes, of which 4,894 are protein-coding genes. Comparison of Conditions C vs. D, in which only the temperature of incubation was different (0 °C vs. 30 °C), resulted in significant alteration of transcript levels of a total of 429 genes, or approximately 8.7% of the protein-coding transcriptome, with 153 and 276
Expression differences due to temperature and atmospheric composition. Comparison of Conditions B vs. D, which differed both in temperature (0 °C vs. 30 °C) and atmospheric gas composition (anoxic CO₂ vs. N₂/O₂), revealed differential expression of 708 total transcripts, or ~14.5% of the protein-coding transcriptome, with 209 and 499 transcripts being up- and down-regulated, respectively (Table 2). An Excel file listing the differentially expressed transcripts found in Condition B vs. D is presented in Supplemental Table S2.

Expression differences due to temperature alone (A vs. B). Comparison of the transcriptome from Condition A (0.7 kPa) vs. B (~101 kPa) revealed significant differential expression of 184 genes, or ~3.8% of the 5302 protein-coding genome, of which 178 were up-regulated and 6 were down-regulated under low pressure (Supplemental Table S5). Examination of the data sets failed to identify specific genes which might be reasoned to facilitate growth at low pressure. Indeed, the analysis revealed that among the most strongly up-regulated genes were those involved in transport and utilization of various sugars (lactose, arabinose, maltose, galactose, or general α-glucosides) or the polyol m-inositol. It should be noted that while TSA medium contains glucose at 0.25% final concentration, none of these other sugars are present in the medium. Of all the environmental parameters tested in our experiments, only low pressure is not encountered in nature on Earth, suggesting that the up-regulation of this plethora of carbohydrate utilization systems at 0.7 kPa is likely maladaptive in S. liquefaciens. This may partly explain why S. liquefaciens grew so slowly and to such a low cell density when cultivated under Condition A (Table 1). Examination of the down-regulated genes showed that the most strongly affected were involved in transport of sulfate or the sulfur-containing amino acid cystine (Supplemental Table S5). Again, the possible relevance of the lowered expression of these genes to growth at low pressure would be speculative at best.

Expression differences due to pressure alone (A vs. B). Comparison of the transcriptome data from Condition A (0.7 kPa) vs. B (~101 kPa) revealed significant differential expression of 184 genes, or ~3.8% of the S. liquefaciens protein-coding genome, of which 178 were up-regulated and 6 were down-regulated under low pressure (Supplemental Table S5). Examination of the data sets failed to identify specific genes which might be reasoned to facilitate growth at low pressure. Indeed, the analysis revealed that among the most strongly up-regulated genes were those involved in transport and utilization of various sugars (lactose, arabinose, maltose, galactose, or general α-glucosides) or the polyol m-inositol. It should be noted that while TSA medium contains glucose at 0.25% final concentration, none of these other sugars are present in the medium. Of all the environmental parameters tested in our experiments, only low pressure is not encountered in nature on Earth, suggesting that the up-regulation of this plethora of carbohydrate utilization systems at 0.7 kPa is likely maladaptive in S. liquefaciens. This may partly explain why S. liquefaciens grew so slowly and to such a low cell density when cultivated under Condition A (Table 1). Examination of the down-regulated genes showed that the most strongly affected were involved in transport of sulfate or the sulfur-containing amino acid cystine (Supplemental Table S5). Again, the possible relevance of the lowered expression of these genes to growth at low pressure would be speculative at best.

Principal component analysis. Because the datasets generated from our RNA-seq experiments consisted of hundreds of genes, we turned to Principal Component Analysis (PCA) to assist us in assessing (i) the reproducibility of the replicates within a Condition, and (ii) whether different treatments result in different groupings. PCA was performed on the 12 datasets, and 4 distinct population clusters were identified which coincided with the 4 environmental conditions tested (Fig. 3). In the PCA, the first and second principal components explained 37% and 24% of the variance, respectively. In each population cluster, the three replicate samples grouped rather tightly, indicating good agreement among replicates for each condition. From inspection of the PCA plot it could be seen that Conditions A, B, and C differed markedly from Condition D, the laboratory control. However, Conditions A and B, which differed in pressure (0.7 kPa vs. ~101 kPa, respectively) clustered rather close to one another, indicating a relatively low degree of gene expression difference (Fig. 3).

Assignment of KEGG categories. Genes whose transcripts were identified as significantly up- or down-regulated were further categorized and assigned to pathways as defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG)19. Up- and down-regulated transcripts are presented in Figs 4 and 5 respectively. Visual inspection of the data revealed that genes up-regulated under all Conditions A, B, and C included: transporters (ABC and PTS transporters); genes involved in translation (ribosomes and their biogenesis, biosynthesis of both tRNAs and aminoacyl-tRNAs); DNA repair and recombination; and non-coding RNAs (Fig. 4). Genes down-regulated under all Conditions A, B, and C included: transporters (mostly ABC transporters); flagellar and motility proteins; genes involved in phenylalanine metabolism; transcription factors; and two-component systems (Fig. 5). Visual examination of the KEGG pathway data failed to identify specific functions that might be postulated to be responsible for the ability of S. liquefaciens to grow, or not, under a particular Condition.

Adaptation of microbes to pressure changes. Of the environmental conditions used in this study, low temperature and anaerobic atmosphere can be found on Earth; only low pressure is a parameter unique to Mars, and the present study is the first to measure the transcriptomic response of a bacterium to low pressure.
comparable to that prevailing at the martian surface (0.7 kPa). To date, only one prior study has been published in which the transcriptomic response of a microorganism to low pressure exposure has been measured, that of the Gram-positive bacterium *Bacillus subtilis* grown at 5 kPa and 27 °C in Earth-normal 80% nitrogen/20% oxygen atmosphere31. In that study, exposure to low pressure led to up-regulation of transcripts from several global regulons (AbrB, CcpA, CodY, Fur, IolR, ResD, Rok, SigH, and Spo0A). Notably, the highest number of up-regulated genes, 86, belonged to the SigB-mediated General Stress Response (GSR) regulon31. In Gram-negative bacteria, the GSR is controlled by RNA polymerase containing the sigma factor RpoS 32. In the present study, we did not observe significant up-regulation of expression of *rpoS* or RpoS-dependent GSR genes by *S. liquefaciens* exposed to simulated Mars conditions.

On the other end of the pressure spectrum, recent studies have reported the global transcriptional responses of piezophilic microorganisms to changes in pressure33–35. The closely related piezophiles *Desulfovibrio hydrothermalis* and *D. piezophilus* each grow at an optimum pressure of 10 MPa. Growth of the organisms at 0.1, 10, and 26 MPa resulted in alterations in expression of genes involved in lactate oxidation, energy metabolism, and amino acid biosynthesis33,35, but very few homologous genes were found to be differentially expressed in both organisms. Likewise, a study comparing the transcriptomic response of two closely related species, *Thermococcus barophilus* and *T. kodakarensis*, to changes in hydrostatic pressure failed to identify transcriptional responses in common34.

The previous studies cited, as well as the present study, were conducted with the intent of searching for new insights into the molecular mechanisms responsible for microbial adaptation to alterations in their pressure environment. In all cases, the sets of genes observed to be altered by pressure appeared to be organism-specific; no shared “pressure-responsive” genes or pathways have been discovered in the studies cited above. This finding parallels numerous studies which have tested explicitly for effects on the transcriptome of 2 or more environmental stressors acting simultaneously. Such studies have uncovered complex effects on gene expression that would not have been predicted from single stressor treatments. Furthermore, exposure to extreme environments can provoke maladaptive responses36. Because *S. liquefaciens*, like all other Earth organisms, has never been exposed to a low pressure of 0.7 kPa during its entire evolutionary history, it is not surprising that exposure to low pressure (Condition A vs. B), resulted in the up-regulation of a number of genes for utilization of carbohydrates not present in the medium, suggesting a maladaptive response to low pressure. What was surprising was that growth of *S. liquefaciens* at low pressure was not the result of a major rearrangement in its transcriptome, as visualized by the PCA plot (Fig. 3). This observation highlights the point that multiple interacting variables affect the transcriptome in ways that are neither intuitive nor predictable.

Relevance to the potential growth of *S. liquefaciens* on Mars. The Martian surface is bathed in numerous biocidal factors among which UV irradiation, low water activity, low pressure, low temperature, and oxidizing conditions predominate37. Despite these harsh conditions, a few studies have demonstrated that as many as 31 bacterial species, including *S. liquefaciens*, are capable of active metabolism and growth under 0.7 kPa, 0 °C, and CO₂-enriched anoxic conditions if cells are incubated in stable UV-protected, hydrated, and nutrient-rich environments15–17. The current study is the first to characterize up- and down-regulated transcripts at 0.7 kPa in a bacterium that may be plausibly transported to Mars (*Serratia* spp. have been recovered from spacecraft surfaces prior to launch38–39); thus our results begin to reveal how at least one terrestrial hypobacterium might be able to grow on Mars. The key for its success will be whether relevant metabolic pathways can
remain active under 0.7 kPa, 0 °C, and CO₂-dominated anoxic atmosphere, and whether cells can be dispersed into hydrated niches containing usable nutrients.

Although organic compounds have been directly detected in Martian regolith, the exact composition of the in situ organics remains unsolved because of the degradation of the organics by perchlorate salts during thermal pyrolysis of surface fines. However, it is now accepted that in situ organics persist in the shallow subsurface on Mars. Furthermore, approx. $2.4 \times 10^5$ kg of meteoritic carbon is accreted annually to Mars in the form of small carbonaceous chondrites and interplanetary dust particles (IDPs). The composition of carbonaceous chondrites and IDPs have been extensively studied, and a comparison between accreted organics to Mars and the up-regulated pathways for S. liquefaciens described here for 0.7 kPa Mars simulations suggest that at least some common metabolic pathways may remain active on the surface of Mars in which accreted organics might be accessible. For example, KEGG analyses indicated that several amino acid, purine, and sugar carbohydrate pathways were up-regulated at 0.7 kPa (Fig. 4), suggesting that these metabolic pathways might remain active on Mars, and that cells of S. liquefaciens might be able to utilize similar classes of organics (i.e., aldehydes, amino acids, ketones, and purines) found in IDPs and carbonaceous chondrites. Our results are consistent with the hypothesis that some terrestrial bacteria are capable of metabolism and growth in stable UV-protected and hydrated niches on Mars, and that accreted or in situ organics may provide the required nutrients for heterotrophic metabolism. However, more research is required to examine the malleability of the transcriptome of S. liquefaciens and other hypobarophiles to fluctuating conditions on the Martian surface.

Finally, the habitability of any martian landing site will be a key consideration for future robotic and crewed spacecraft in which either life-detection experiments will be conducted or human exploration traverses completed. If: (i) hypobarophilic microorganisms are present on spacecraft or payload surfaces prior to launch; (ii) landing sites are near terrains that might harbor an extant Mars microbiota or support the growth of terrestrial

Figure 4. Up-regulated genes sorted by KEGG orthology. The number of genes of a particular KEGG pathway are depicted as a fraction of the total number of genes classified in that pathway in the genome of S. liquefaciens. See Table 1 for description of the conditions.
microbes (i.e., defined as Special Regions); and (iii) metabolic pathways remain active at 0.7 kPa that can utilize in situ or accreted organics on Mars, then the risk of the forward contamination of such sites will remain high.

Materials and Methods

Bacterial strain and medium. The bacterial strain used in this study was *Serratia liquefaciens* strain ATCC 27592 obtained from the American Type Culture Collection, Manassas, VA USA. Its complete genome sequence has been determined and is deposited in the National Center for Biotechnology Information (NCBI) GenBank database under accession number CP006252.1. Medium used throughout was Tryptic Soy Agar (TSA) (BD Difco, Franklin Lakes, N J USA).

Growth conditions. The experimental conditions used are described in Table 1. In brief, simulated Mars conditions (Condition A) were chosen based on the near-ubiquitous extent of low pressure between 0.7 and 1.0 kPa at the surface, the nominal CO₂-dominated (96%) atmosphere, and the requirement to maintain a stable liquid environment near the triple-point of water at 0 °C and 0.7 kPa. The control conditions B, C, and D were chosen in order to discriminate the effects of pressure, gas composition, and temperature, respectively, compared to the Martian conditions tested. Although numerous other environmental and geochemical conditions might be present at the surface of Mars (e.g., diel temperature swings, low water activity, UV irradiation, high salt or perchlorate concentrations, oligotrophic nutrient regimes in the regolith) the first-order Mars simulations used here were chosen to create a stable hydrated and nutrient-rich environment in order to characterize transcriptomic changes in *S. liquefaciens* under a core set of three common environmental conditions on the Martian surface. The simulated Martian conditions used are consistent with models that suggests stable liquid water may occur on present-day Mars.
Cultures were prepared in triplicate. For each replicate at each growth condition, the number of TSA plates indicated in Table 1 was inoculated with cells from a freshly-prepared culture grown overnight in a laboratory incubator at 30 °C. Cultures were incubated in a desiccator/low-pressure control system as described previously16,17. Briefly, TSA plates seeded with S. liquefaciens cells were placed into a 4-L polycarbonate desiccator (model 08-642-7, Fisher Scientific, Pittsburg, PA, USA), surrounded by four anaerobic pouches (Remel Anaerobic Pack sachets, Fisher Scientific), the desiccator top attached to the desiccator body, and the lab atmosphere flushed with filter-sterilized, ultra-high purity CO2 gas for 3–4 min, resulting in an atmosphere of essentially 100% CO2. The desiccator was sealed, connected to an externally mounted pump and low-pressure control system (model PU-842, KNF Neuberger, Trenton, NJ, USA), and placed within a microbial incubator set at 0°C.

The equilibration process to stabilize the cultures at low-pressure conditions without boiling the media required approximately 60 min. The desiccators were pumped down to 10, 5, 2.5, and 0.7 kPa sequentially for approx. 15 min per time-step. The cumulative 60-min equilibration time allowed the cultures to outgas laboratory air or dissolved CO2 while also simultaneously cooling the media slowly to 0°C. At 7-day intervals, the desiccators were vented to lab air and exhausted anaerobic pouches were replaced with fresh ones. Anaerobic pouches similar to those described above have been shown to remove O2 to a concentration of ≤0.1% within 1 h in small closed containers49; this concentration closely matches the concentration of O2 in the martian atmosphere (0.145%)50.

After incubation, cells were removed from agar surfaces by rubbing with a sterile glass rod and suspended in sterile, ice-cold PBS buffer51. Cell suspensions were immediately centrifuged (7,000 × g, 0 °C, 20 min) and the cell pellets stored at −70°C until use.

Isolation of total RNA. Total RNA was extracted from cell pellets and treated with RNase-free DNase I using the RiboPureTM RNA Purification Kit (Thermo Fisher Scientific, Waltham, MA) following the manufacturer’s protocol. Total RNA content in samples was determined using a Qubit fluorometer (Invitrogen, Thermo Fisher Scientific Inc, Waltham, MA). Sample quality was measured using the RNA 6000 Nano Chip and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The RNA Integrity Number (RIN)52 values of the samples are presented in Table 1.

RNA-seq and data analysis. RNA samples were shipped on dry ice to the Hudson Alpha Institute for Biotechnology, (Huntsville, AL) where they were subjected to ribosomal RNA reduction, library preparation, and multiplex 50-nucleotide single-end sequencing on an Illumina HiSeq2500 instrument. The raw Illumina sequences were imported into the University of Florida’s High Performance Research Computer HiPerGator 2 platform as.fastq files for further processing. Low-quality base calls were trimmed from the sequences using fastx-trimmer version 0.0.14 with a quality threshold of 33. Trimmed reads were mapped to the S. liquefaciens strain ATCC 27592 genome (NCBI RefSeq NC_021741.1)28 using bowtie2 version 2.3.252, and the aligned sequence files were imported into the University of Florida’s High Performance Research Computer HiPerGator 2 platform as.fastq files for further processing. Low-quality base calls were trimmed from the sequences using fastx-trimmer version 0.0.14 with a quality threshold of 33. Trimmed reads were mapped to the S. liquefaciens strain ATCC 27592 genome (NCBI RefSeq NC_021741.1)28 using bowtie2 version 2.3.252, and the aligned sequence files were imported into the HTSeq version 0.6.153 for read counting. All differential expression analyses were performed in R version 3.4.2 using the package limma54–56. Per limma recommendation, transcripts with less than 10 total counts across all samples were removed. Gene counts were normalized using the trimmed mean of M values (TMM) method, and the normalized counts were transformed using the built-in voom method. The log2-fold change values for each transcript were determined using limma’s default eBayes method and the P values were adjusted for multiple testing using the Benjamini-Hochberg method57. Genes were considered to be differentially expressed if they exhibited at least a 4-fold (i.e., a log2 value >2) difference in transcript levels between conditions with a Benjamini-Hochberg adjusted P value less than 0.01. Principal Component Analysis (PCA) was performed on the normalized gene counts using the built-in R package stats, and the loading scores for the first two principal components were plotted in R. The processed read data are available in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database under accession number GSE120390.

Functional enrichment analysis was performed using BLAST2GO version 4.1.958. FASTA sequences for differentially expressed genes were retrieved from the PATRIC database59 using the KEGG locus tags associated with each gene. A BlastX-fast program was run for all FASTA sequences against the non-redundant database with an α-proteobacterial (taxa: 28211, Alphaproteobacteria) filter. Default mapping and annotation parameters were used, and Blast expectation values were set at a threshold of 1.0E–3. Annotation configurations were run with the default parameters and successfully annotated hits were mapped to Gene Ontology (GO) terms and pathways in the KEGG database60,61.

References
1. Chyba, C. F. & Hand, K. P. In Annu. Rev. Astron. Astrophys. Vol. 43 31–74. (Annual Reviews, 2005).
2. Cockell, C. S. et al. Habitability: A Review. Astrobiology 16, 89–117, https://doi.org/10.1089/ast.2015.1295 (2016).
3. Fajardo-Cavazos, P., Schuerger, A. C. & Nicholson, W. L. Testing interplanetary transfer of bacteria between Earth and Mars as a result of natural impact phenomena and human spaceflight activities. Acta Astronaut. 60, 534–540 (2007).
4. Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. & Setlow, P. Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiol. Mol. Biol. Rev. 64, 548–572, https://doi.org/10.1128/mmbr.64.3.548-572.2000 (2000).
5. Rummel, J. D. et al. A new analysis of Mars “Special Regions”: findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2). Astrobiology 14, 887–968, https://doi.org/10.1089/ast.2014.1227 (2014).
6. Nicholson, W. L. Ancient micrornauts: interplanetary transport of microbes by cosmic impacts. Trends Microbiol. 17, 243–250, https://doi.org/10.1016/j.tim.2009.03.004 (2009).
7. Nicholson, W. L., Schuerger, A. C. & Race, M. S. Migrating microbes and planetary protection. Trends Microbiol. 17, 389–392, https://doi.org/10.1016/j.tim.2009.07.001 (2009).
8. Munteanu, A., Uivarosi, V. & Andries, A. Recent progress in understanding the molecular mechanisms of radioresistance in Deinococcus bacteria. Extremophiles 19, 707–719, https://doi.org/10.1007/s00792-015-0759-9 (2015).
9. Hirokoshi, K. et al. Extremophiles Handbook. 608 pp. (Springer, 2011).
27. Moissl, C.

23. Machado, S. G.

15. Nicholson, W. L., Krivushin, K., Gilichinsky, D. & Schuerger, A. C. Growth of  

17. Schuerger, A. C., Ulrich, R., Berry, B. J. & Nicholson, W. L. Growth of  

33. Amrani, A.

26. Ghosh, S., Osman, S., Vaishampayan, P. & Venkateswaran, K. Recurrent isolation of extremotolerant bacteria from the clean room  

32. Landini, P., Egli, T., Wolf, J. & Lacour, S. sigmaS, a major player in the response to environmental stresses in  

19. Engelhart, S.

20. Mossad, S. B. The world's first case of  

31. Waters, S. M., Robles-Martínez, J. A. & Nicholson, W. L. Exposure of  

34. Vannier, P., Michoud, G., Oger, P., Marteinsson, V. & Jebbar, M. Genome expression of  

14. Berry, B. J., Jenkins, D. G. & Schuerger, A. C. Effects of simulated Mars conditions on the survival and growth of  

12. Moores, J. E., Smith, P. H., Tanner, R., Schuerger, A. C. & Venkateswaran, K. J. The shielding effect of small-scale martian surface  

13. Schuerger, A. C. & Nicholson, W. L. Interactive effects of hypobaria, low temperature, and CO2 atmospheres inhibit the growth of  

11. Nicholson, W. L., Schuerger, A. C. & Setlow, P. The solar UV environment and bacterial spore UV resistance: considerations for  

26. Ghosh, S., Osman, S., Vaishampayan, P. & Venkateswaran, K. Recurrent isolation of extremotolerant bacteria from the clean room  

19. Engelhart, S. et al. Severe Serratia liquefaciens sepsis following vitamin C infusion treatment by a nutraceutical practitioner.  

Mossad, S. B. The world's first case of Serratia liquefaciens intravascular catheter-related supplicative thrombophlebitis and native valve endocarditis.  

Pinna, A., Usai, D., Sechi, L. A., Carta, A. & Zanetti, S. Detection of virulence factors in Serratia strains isolated from contact lens-associated corneal ulcers.  

Haq, I., Kumar, S., Kumari, V., Singh, S. K. & Raj, A. Evaluation of bioremediation potential of ligninolytic Serratia liquefaciens for detoxification of pulp and paper mill effluent.  

Ghosh, S., Osman, S., Vaishampayan, P. & Venkateswaran, K. Recurrent isolation of extremotolerant bacteria from the clean room where Phoenix spacecraft components were assembled.  

Moissl, C. et al. Molecular bacterial community analysis of clean rooms where spacecraft are assembled.  

Nicholson, W. L. Complete Genome Sequence of Serratia liquefaciens Strain ATCC 27592. Genome Announc. 1, e00548–00513, https://doi.org/10.1128/genomeA.00548–13 (2013).  

Markowitz, V. M. et al. IMG: the integrated microbial genomes database and comparative analysis system.  

Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes.  

Waters, S. M., Robles-Martínez, J. A. & Nicholson, W. L. Exposure of Bacillus subtilis to low pressure (5 kPa) induces several global regulators including the sigB-mediated General Stress Response.  

Landini, P., Egli, T., Wolf, J. & Lacour, S. sigmaA, a major player in the response to environmental stressors in Escherichia coli: role, regulation and mechanisms of promoter recognition.  

Amrani, A. et al. Deciphering the adaptation strategies of Desulfovibrio piezophilus to hydrostatic pressure through metabolic and transcriptional analyses.  

Vannier, P., Michoud, G., Oger, P., Marteinsson, V. & Jebar, M. Genome expression of Thermococcus barophilus and Thermococcus kodakarenensis in response to different hydrostatic pressure conditions.  

Nicholson, W. L. Exposure of Bacillus subtilis to low pressure (5 kPa) induces several global regulators including the sigB-mediated General Stress Response.  

DeRousse, M. B. & Kelly, M. V. Plastic and evolved responses to global change: what can we learn from comparative transcriptomics?  

Ming, D. W. et al. Volatile and organic compositions of sedimentary rocks in Yellowknife Bay, Gale crater, Mars.  

Navarro-Gonzalez, R., Vargas, E., de la Rosa, J., Raga, A. C. & McKay, C. P. Reanalysis of the Viking results suggests percholrate and  

Clavin, D. et al. Evidence for percholrates and the origin of chlorinated hydrocarbons detected by SAM at the Rocknest aeolian deposit in Gale Crater.  

Flyn, G. J. The delivery of organic matter from asteroids and comets to the early surface of Mars. Earth, Moon & Planets 72, 469–474, https://doi.org/10.1007/bf01175511 (1996).  

Sephton, M. A. Organic compounds in carbonaceous meteorites. Nat. Prod. Rep. 19, 292–311, https://doi.org/10.1039/b00775g (2002).  

Sephton, M. A. & Botta, O. Recognizing life in the Solar System: guidance from meteoritic organic matter. Int. J. Astrobiol. 4, 269–276, https://doi.org/10.1017/s147355040500806(2005).  

Clemett, S. J., Maechling, C. R., Zare, R. N., Swan, P. D. & Walker, R. M. Identification of complex aromatic molecules in individual interplanetary dust particles. Science 262, 721–725, https://doi.org/10.1126/science.262.5134.721 (1993).  

Gómez-Elvira, J. et al. Curiosity's rover environmental monitoring station: Overview of the first 100 sols. J. Geophys. Res. Planets 119, 1680–1688 (2014).  

Hess, S. L., Ryan, J. A., Tillman, J. E., Henry, R. M. & Leovy, C. B. The annual cycle of pressure on Mars measured by Viking Landers  

Malahoff, P. R. et al. Abundance and isotopic composition of gases in the martian atmosphere from the Curiosity rover. Science 341, 263–266, https://doi.org/10.1126/science.1237966 (2013).  

Haberle, R. M. et al. On the possibility of liquid water on present-day Mars. J. Geophys. Res. 106, 23317–23326 (2001).  

Lobitz, B., Wood, B. L., Averner, M. M. & McKay, C. P. Use of spacecraft data to derive regions on Mars where liquid water would be stable. Proc Natl Acad Sci USA 98, 2132–2137, https://doi.org/10.1073/pnas.031581098 (2001).
49. Van Horn, K. G., Warren, K. & Baccaglini, E. J. Evaluation of the AnaeroPack system for growth of anaerobic bacteria. J. Clin. Microbiol. 35, 2170–2173 (1997).
50. Nicholson, W. L. & Setlow, P. In Molecular biological methods for Bacillus. (eds C. R. Harwood & S. M. Cutting) 391–450 (J. Wiley & Sons, 1990).
51. Schroeder, A. et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 7, 3, https://doi.org/10.1186/1471-2199-7-3 (2006).
52. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10, R25, https://doi.org/10.1186/gb-2009-10-3-r25 (2009).
53. Anders, S., Pyl, P. T. & Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169, https://doi.org/10.1093/bioinformatics/btu638 (2015).
54. Ritchie, M. E. et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43, e47, https://doi.org/10.1093/nar/gkv007 (2015).
55. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 15, R29, https://doi.org/10.1186/gb-2014-15-2-r29 (2014).
56. Liu, R. et al. Why weight? Modelling sample and observational level variability improves power in RNA-seq analyses. Nucleic Acids Res 43, e97, https://doi.org/10.1093/nar/gkv412 (2015).
57. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Royal Statist. Soc. B 57, 289–300 (1995).
58. Götz, S. et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res 36, 3420–3435, https://doi.org/10.1093/nar/gkn176 (2008).
59. Wattam, A. R. et al. Assembly, annotation, and comparative genomics in PATRIC, the All Bacterial Bioinformatics Resource Center. Methods Mol Biol 1704, 79–101, https://doi.org/10.1007/978-1-4939-7463-4_4 (2018).
60. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44, D457–462, https://doi.org/10.1093/nar/gkv1070 (2016).
61. Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. & Morishima, K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45, D353–D361, https://doi.org/10.1093/nar/gkw1092 (2017).

Acknowledgements
This work was funded by the National Aeronautics and Space Administration (NASA) Planetary Protection grant NNX12AJ84G.

Author Contributions
P.F.-C., W.L.N. and A.C.S. conceived and performed the experiments. K.M.M. and M.D.M. performed the bioinformatics data processing. W.L.N. performed the biological analysis. All authors participated in writing the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-33140-4.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and indicate if changes were made. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018