Single cell whole genome amplification in optofluidic platform and sequencing assessment from the Biology and Mars Experiment (BIOMEX)

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Introduction: Microbial genome sequencing in low biomass settings such as in extreme conditions could lead to crucial findings in astrobiological pursuits. Standard sequencing requires high biomass and averages over genomically heterogeneous populations, concealing the valuable information hidden within very few cells. Single cell whole genome sequencing (SC-WGS) enables the identification of microbes with low representation often neglected or undetectable in traditional studies[1]. Using SC-WGS, detection of rare mutation events in single cells is possible, allowing for mutagenic and detailed evolutionary work. Here, we use an optofluidic platform to obtain single cells of *Gleocapsa sp*., *Sphaerocystis* sp. Arctic strain CCCryo 101-99 and *Nostoc* sp. Antarctic strain CCCryo 231-06 for SC-WGS to identify the genomic variation among the cells exposed to the Earth condition, the simulated Martian condition on Earth, and the simulated Martian condition on the International Space Station, and to investigate microbial responses to space radiation on a single cell level.

Experimental platform: The platform integrates microscopy, optical tweezers and a microfluidic device[2]. The microfluidic device is capable of chamber formation and high-throughput sample processing with minimal cross-contamination (Fig.1(a)). Single cells can be trapped by laser tweezers, moved into a chamber without physical contact and amplified for SC-WGS (Fig.1(b)). Suspension of *Gleocapsa sp*., *Sphaerocystis* sp. and *Nostoc* sp. exposed to the aforementioned conditions were introduced into a microfluidic device respectively (Fig.1(c)). We developed effective single cell lysis protocol for on-chip whole genome amplification and reached 100% success (obtained > 25 ng DNA) for all three species[3].

Results: A total of 108 samples (27 experimental samples, 4 replicates from each sample, and 6 negative controls) were sent for Illumina sequencing in a HiSeq 4000 platform. Consensus genomes were created for all 3 organisms. Near-complete recovery was achieved for *Nostoc* sp., while only partial recovery was achieved for the other 2 organisms. All 3 consensus genomes matched known rRNA sequences for these specific strains. Using conserved protein sequences, the *Nostoc* sp. was phylogenetically placed as a sibling species to *N. punctiforme* PCC 73102 = ATCC 29133. For cf. *Gloeocapsa* OU-20, there was enough genomic information to putatively place it as a member of the genus *Aliterella*[4]. The best efficiency (combined purity and coverage) was observed in samples in BG11 medium, for both flight samples and ground controls. Lunar and Martian analogue samples were able to display a high degree of purity, but overall poor degree of coverage (Fig. 2). When comparing *Nostoc* sp. flight vs. ground isolates in BG11 medium, we observed variants in the sequence for the biofilm-associated filamentous hemagglutinin, as well as the D1 protein of photosystem II. Multiple variants in transposases were also observed. Further functional and biological analysis of the sequencing data is ongoing, which will allow for an unparalleled study of single cell mutagenic events within a population and observation of the raw rate of mutation events that a single cell experiences under various conditions and shed light on microbial responses to space radiation.

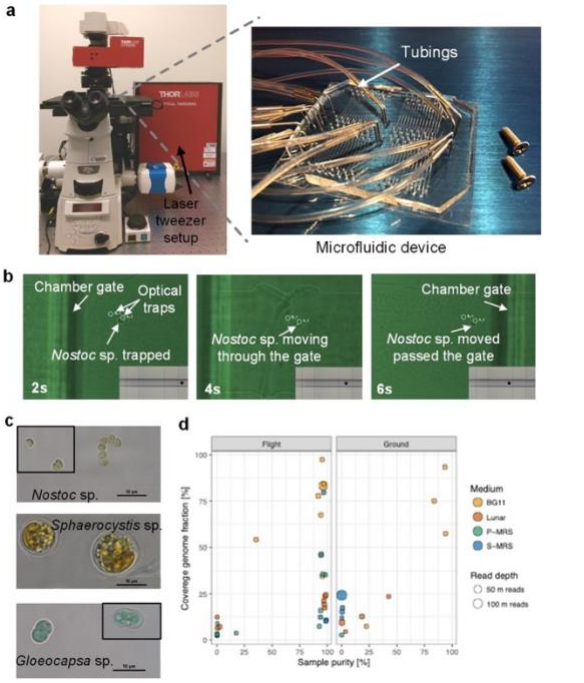

Fig.1 (a) An optofluidic platform with microscope, laser tweezers and microfluidic device. (b) Moving single cell into a microchamber by laser tweezer. (c) Images of cell species. (d) Sample purity and genome coverage of BIOMEX single-cell samples.

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