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Note

Microbial survival in space shuttle crash

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Abstract

A slow growing, heat resistant bacterium, identified by 16S rRNA gene sequencing as *Microbispora sp*, was recovered from the wreckage of the ill-fated space shuttle Columbia (STS-107). As this organism survived disintegration of the space craft, heat of reentry, and impact, it supports the possibility of a natural mechanism for the interplanetary spread of life by meteorites.

Keywords: Exobiology; Meteorites

Panspermia, the theory of interplanetary spread of life, has been an intriguing concept of astrobiology (exobiology). In 1996, microscopic examination of bacteria-shaped structures in martian Meteorite ALH84001 (McKay et al., 1996) provided the first experimental suggestion of panspermia. Similar objects of potential biological origin were also observed in the Tatahouine meteorite (Gillet et al., 2000). Assuming that an organism remained viable following ejection from the planet of origin and exposure to vacuum and solar radiation (Nicholson et al., 2005) during space travel, it would still need to survive the heat of atmospheric entry and force of impact. Here, we report on the survival of a microorganism, *Microbispora sp*, from the payload of the ill-fated space shuttle Columbia.

The microbiology experiment flown on Columbia was originally designed to study interactions in unattached (planktonic) and surface-adherent (biofilm) populations of three organisms, *Escherichia coli* ATCC 23848, *Chromobacterium violaceum* ATCC 12472, and *Pseudomonas aeruginosa* PAO-1. A mixed suspension of these organisms, along with sterile polycarbonate membranes, was then shipped to Instrumentation Technology Associates (ITA) (Exton, PA), whose personnel loaded the samples into a Dual Materials Dispersion Apparatus (DMDA), commercial payload (McLean et al., 2001). The DMDA was then transported to Kennedy Space Center, Florida and flown on space shuttle mission STS-107.

STS-107 launched on January 16, 2003. During reentry on February 1, 2003, the spacecraft disintegrated. At loss of signal the shuttle was traveling at Mach 18.16 (9700 km h^{-1}) at an altitude of 200,767 ft (61.2 km) (Over et al., 2004). The DMDA payload was recovered intact in a parking lot (Fig. 1A) and transported to NASA facilities at Kennedy Space Center in Florida. Access to the payload was obtained on May 6, 2003. Upon opening, the DMDA internal components were intact, which should eliminate the possibility of contamination during recovery and storage. While the outer aluminum shell was charred (Fig. 1A), the inner acetal polymer components (Fig. 1B) exhibited only minor melting on one edge, indicating brief, transient exposure to temperatures approximating 175 °C. Trace amounts of H_2O were present. Sampling consisted of aseptic removal of all liquids, and rinsing sample wells with sterile Luria–Bertani (LB) broth (to attempt to revive any surviving microorganisms), and TE buffer (10 mM tris(hydroxymethyl) aminomethane, 1 mM EDTA (ethylene diamine tetra-acetate), pH 8.0) (to remove DNA). On site contamination was monitored by running process controls in which sterile petri dishes were rinsed with LB broth and TE buffer in an attempt to recover contaminating microorganisms and nucleic acids. Samples were flown back to Texas and incubated at 30 °C. Polymerase chain reaction (PCR) and sequencing of the 16S rRNA gene was also performed using eubacterial primers 27F and 1492R (Gillan et al., 1998). Based on extended (6 months) culturing and PCR analyses (for DNA detection), no evidence of contamination was found in the process controls, in any of the solutions, nor uninoculated media. While none of the original bacterial inoculum was detected, a slow growing organism was observed from a culture prepared from an LB rinse of the DMDA payload. This organism was sent to MIDI Labs (Newark, DE) for partial (500 bp) 16S rRNA gene sequencing. Sequence information was deposited with GenBank (National Library of Medicine, Bethesda MD) and given accession number AY701903. Phylogenetic analysis using distance-based, neighbor-joining and bootstrap supports in PAUP* 4.01b software (Swofford, 2002) identified this organism as *Microbispora sp* (Fig. 1C). No other organisms were isolated or detected.

In contrast to higher lifeforms, Bacteria and Archaea (sometimes referred to as Archaebacteria) can grow and survive under a wide range of adverse environmental conditions. Consequently, microorganisms are considered to be the most likely candidates for exobiology (Marion et al., 2003). During space travel, organisms would be in high vacuum, lack liquid water, and would be exposed to temperature extremes as well as elevated and potentially lethal doses of radiation [reviewed in (Nicholson et al., 2005)]. Although microgravity would also be encountered during space travel, experimental evidence suggests that

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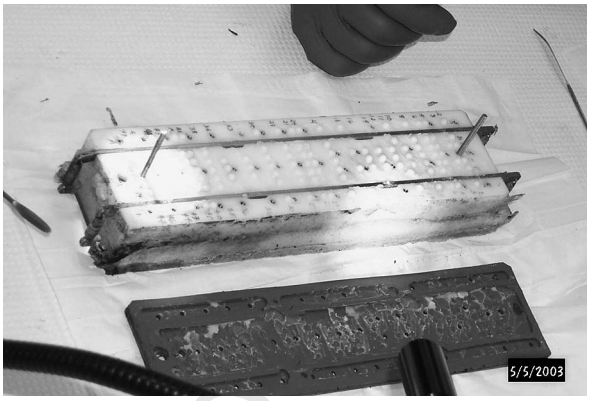
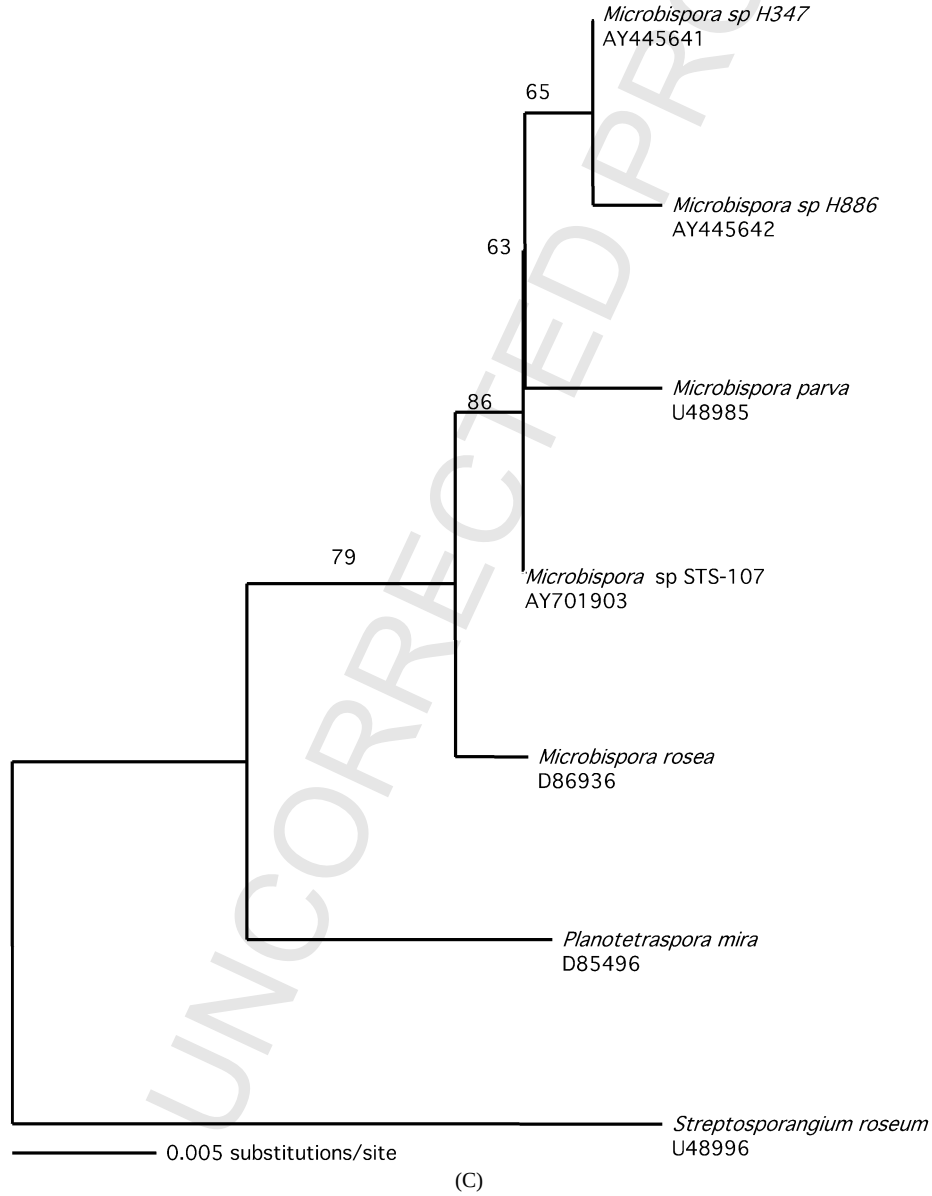
(A)



(B)



(C)

Fig. 1. The DMDA was recovered relatively intact (A) (Over et al., 2004), with slight melting observed on the acetal components (B). Phylogenetic analysis of *Microbispora sp* STS-107 (C) with numbers indicating phylogenetic bootstrap support (Swofford, 2002).

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microorganisms survive and even thrive under reduced gravity (McLean et al., 2001; Song and Leff, 2005). During the brief transit from space, through the atmosphere, to planetary impact, a meteorite would encounter rapidly increasing pressure, heat from atmospheric friction and meteorite ablation, and impact. The velocity of meteorites relative to Earth is estimated to be 12–20 km/s and the atmospheric transit time estimated to be on the order of 10 s (Sears, 1975). Based on studies of mineralogy and thermoluminescence characteristics of iron-containing meteorite samples, Sears (1975) estimated temperatures of 200 °C or greater would penetrate only to a depth of approximately 5–10 mm. To address the possibility of carbonate meteorites not surviving atmospheric passage, Brack et al. (2002) placed a number of sedimentary minerals, including simulated martian regolith (basalt in a gypsum matrix), on the heat shield of a spacecraft, and studied mineralogical changes that occurred during the heat of reentry. While heat-induced changes did occur, these materials, potentially capable of transporting lifeforms, did survive (Brack et al., 2002).

Of these factors, heat is likely going to be the factor that is most likely to affect microbial survival. Forces due to impact would also influence survival. In laboratory experiments, bacteria of the genus *Rhodococcus* exhibited limited survival following an impact of 5.1 km s^{-1} (Burchell et al., 2001). However, viable bacteria are commonly harvested from liquid culture by centrifugal forces approaching 10,000× gravity (Gerhardt and Drew, 1994). Similarly, sudden changes in atmospheric pressure from a high vacuum to full atmospheric pressure are not likely to reduce microbial survival, as such conditions are used during the preparation of freeze-dried (lyophilized) microbial cultures for maintenance of long term viability (Gherna, 1994). Although conditions encountered during meteorite passage through the atmosphere and impact are harsh, experimental evidence to date would support some microbial survival.

Microbispora sp occur in a number of different environments including soil and air, which could account for its presence as an environmental contaminant. Microorganisms have also been recovered from the stratosphere (Wainwright et al., 2003), which may also represent a potential source of *Microbispora sp*. As dry heat tolerance (120 °C) has been used as a culture enrichment technique for this genus (Hayakawa et al., 1991), its survival is understandable. This isolate did not withstand autoclaving (moist heat) in which steam is used to generate temperatures of 121 °C. As the STS-107 accident could not have been forseen, additional experimental controls and replicates were not performed. Consequently, the occurrence of this strain, designated *Microbispora sp* strain STS-107, is attributed to likely environmental contamination of the payload (Pierson, 2001) prior to launch. In conclusion, our findings provide experimental support for biological survival given the atmospheric-passage, heat and impact of a space-borne object, such as might occur during panspermia (McKay et al., 1996).

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