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Mycology studies in space

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Introduction

Fungal species serve as excellent test organisms when studying environmental systems and the influences of environmental parameters on cells, cell organelles, and cell structure. Various taxonomic classes, numerous orders and families categorize the diverse mycological forms representing thousands of fungal species. Fungi show great diversification in cell form and function, producing species that could serve as test organisms to investigate complex and harsh environments.

Many studies have been conducted with fungi on board balloons, earth satellites, and high altitude sounding rockets for evaluations of environmental conditions in space. The Apollo Microbial Ecology Evaluation Device (MEED) space flight hardware permitted the return of the flight package containing microbial cells killed from exposure to specific space parameters, cells modified by space irradiations, and cells unchanged by the space flight. The studies through NASA required the investigation of viability and survival rates of the space flown fungi housed in the MEED. Through many interested scientists, postflight studies still continue on the retrieved fungal isolates housed on board the Apollo MEED. It is through postflight studies that initial changes induced at the cellular level in space can be examined and identified. No space flight mission before the Apollo program gave opportunity to qualitatively and quantitatively examine cellular systems in space after exposure to specific space parameters. The space environmental parameters were measured by the space hardware during the time of fungal cell deployment.

Selected fungal species were placed in the Microbial Ecology Evaluation Device of Apollo and were exposed to various qualitative and quantitative space flight parameters according to the designs and construction of the flight hardware. Two yeast fungi, *Rhodotorula rubra* and *Saccharomyces cerevisiae*, and two filamentous fungi, *Trichophyton terrestre* and *Chaetomium globosum*, were the four species selected from two years of preflight studies that examined all classes of fungi in order to gain maximum postflight data and information on the effects of space on living cellular systems.

Upon retrieval of the organisms at splashdown, the flight hardware was immediately air transported to the Lunar Receiving Laboratory at NASA JSC for unloading and for initial studies on each species. The first group of studies on the four unrelated fungi concerned survival rates at each of several space flight parameters examined. Postflight studies continued on the retrieved organisms for 15 years after Apollo that examined gross changes at the macroscopic level. Several additional postflight studies identified tissue variation, cellular changes, subcellular variation, and changes in the physiology of the organisms according to the selected exposure parameters examined in space. The designed studies on space flown fungal phenotypes were made in comparison to various space flight controls, solar simulation, launch and ground controls.

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Literature review

The early space program included fungi as test organisms in initial studies of space exploration. Pioneering investigators understood exomycological studies could reveal effects of space flight parameters at the cellular level of fungal species. Kovyazin and coworkers [30] noted haploid yeasts previously sensitized with oleic acid were more sensitive to space flight factors than their diploid counterparts. Clark [7] described the influence of heavy particle radiations on *Neurospora crassa* at high altitudes, and recommended heavy shielding to protect manned space vehicles against galactic, cosmic, and solar radiations. Jenkins [25] reported an increase in mutational levels in fungal spores of *Neurospora crassa* flown on board Discoverer 8 and NERV 1 missions. The genetic damage in *N. crassa* was further examined by other workers [9, 39, 40]. *Neurospora* sp. on Gemini 3 showed no significant effects from zero gravity and radiation but a 2-fold increase in the number of chromosomal breaks at all radiation levels after exposure to P^{32} B radiation. Harmful effects of 660 MeV proton irradiation on yeasts were examined by several other scientists [2].

Yeasts flown on board Kosmos 110 proved to be potential sources of proteins, carbohydrates, and vitamins on extended space flights [35]. Gemini 11 mission produced no significant synergistic effects of radiation and zero gravity on asexual spores of a 2-component heterokaryon [10, 11].

Penicillium roqueforti conidia were highly resistant to drying and storage on board Agena and Gemini 9A [18]. Early sounding rocket studies introduced fungi to space parameters for only a few minutes but contradicted previously held predictions that immediate sterilizations of Earth microbes would occur during periods of direct exposure to the space environment [15]. The Arcas sounding rockets carried growing basidiocarps of *Schizophyllum commune* and demonstrated stress and cell rearrangement in young lamellae during suborbital flights [42].

Gemini 11A, Gemini 12 and Agena 8 satellite borne exposure and collection facilities revealed the absence of viable microorganisms on sterile methylcellulose collection surfaces [32]. Zhukova and Kozlova [31] presented evidence indicating that pigmented, non-sporulating yeast cells were among the most highly resistant microorganisms screened for experiments designed to simulate Martian conditions. *Aspergillus niger* cells were able to withstand elevated temperatures while exposed to ultrahigh vacuum conditions of 10^{-8} mm Hg [6]. Cells of *Alternaria* sp. were cultured in an argon atmosphere while *Mucor mucedo*, *Aspergillus niger*, *Botrytis* sp., and *Torula* sp. were among the fungal genera screened in a Martian simulator [38]. In other experiments, Mardres [34] determined the viabilities of selected microorganisms following their exposure to elevated UV flux similar to conditions of the Martian surface.

Martian environment possibly could support growth of unicellular and multicellular organisms [8, 24, 31, 33]. Yeasts, molds, and various Actinomycetes developed normally under conditions of deep hypothermia, N_2 atmospheres, and greatly reduced moisture levels. Yeast cells of *Endomyces magnusii*, mycelial fragments, and fungal spores in the form of aerial propagules survived conditions of extreme cold and dryness [3, 33]. Antarctic Dry Valley ecology, similar to the topography of possible future Mars landing sites, support filamentous fungi and yeasts as noted in current studies [1].

Materials and methods

Vegetative yeast cells, conidia and ascospores of the four selected fungal species were housed in the Apollo Microbial Ecology Evaluation Device (MEED) and were exposed at a 90° angle to the sun for 10 min plus 7 sec in space during the transearth Extra Vehicular Activity (EVA) [44]. Criteria for species selection included the ability to survive constraints of the planned space flight hardware, usefulness of the species for identifying change at the cellular level incurred in space, previous space related research in mycology

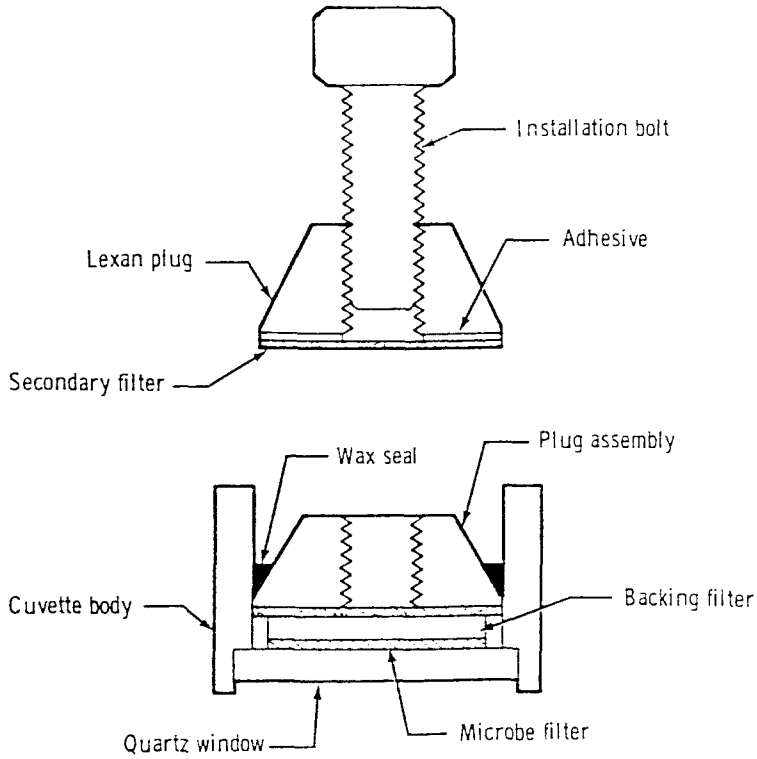

Fig. 1. Cuvette construction of cells housed dry at concentrations to permit a monolayer of yeast cells or fungal spores equal distribution on the microbe filter for UV exposure in space.

related to the proposed studies, and ease of handling the organisms [45, 56]. The role of fungi in space related research was investigated to assist in the selection of species for the Apollo MEED project [13]. Selection of the four fungal test flight species involved a two year preflight study to determine maximum productivity and data reliability of each selected species [51]. Preflight design of experiments attempted to utilize each test flight species in diverse postflight studies.

Flight hardware cuvettes were designed to hold a known number of either dry inoculum or a distilled H_2O suspension of the fungal cells (Figs. 1, 2). Each cuvette had a quartz window, band-pass filter, and neutral density filter for exposure to specific ultraviolet light components of solar irradiation (Figs. 3, 4). Cells were exposed to 254, 280, and 300 nm UV light at various energy levels, full solar light, and no light [52]. Some cells housed in dry cuvettes were vented to space vacuum according to the position of each cuvette in the flight hardware trays (Fig. 5). Additional controls for wild-type cells included ground control cells housed at room temperature, flight control cells housed in the flight hardware with no light exposure, vibration control and solar simulation control cells housed in equipment at NASA JSC to simulate maximum space flight parameters at maximum levels effecting the mycology MEED experiments.

At splashdown of Apollo, the MEED flight hardware (Fig. 6) was returned to the NASA JSC Lunar Receiving Laboratory, Houston TX. Space exposed phenotypes were isolated and maintained on Sabouraud dextrose agar for further testing [44, 48].

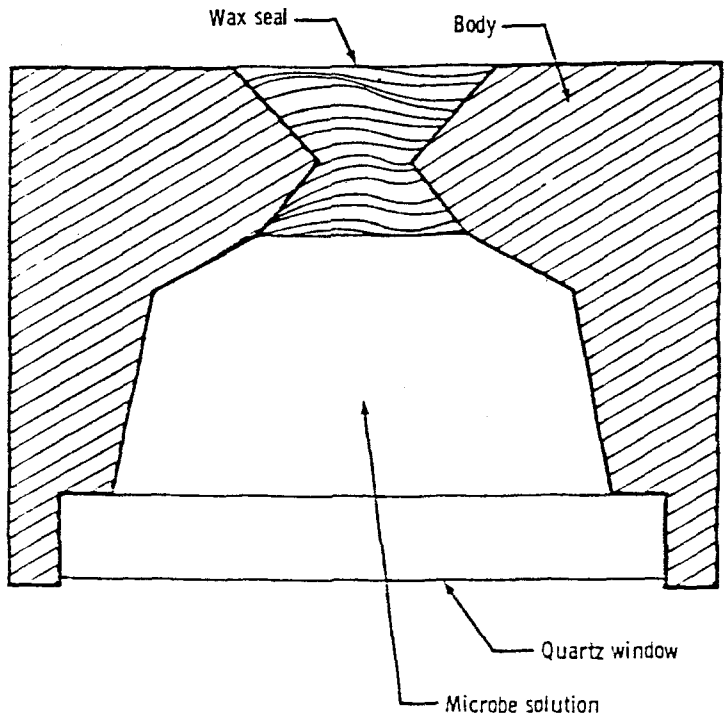

Fig. 2. Cuvette style in which fungal spores and yeast cells were housed in a 0.05 cm^3 volume of water at a cell concentration to permit UV exposure to all cells when deployed in space. Cuvette internal slope was 7° to reduce possible shadowing of fungal cells within chamber.

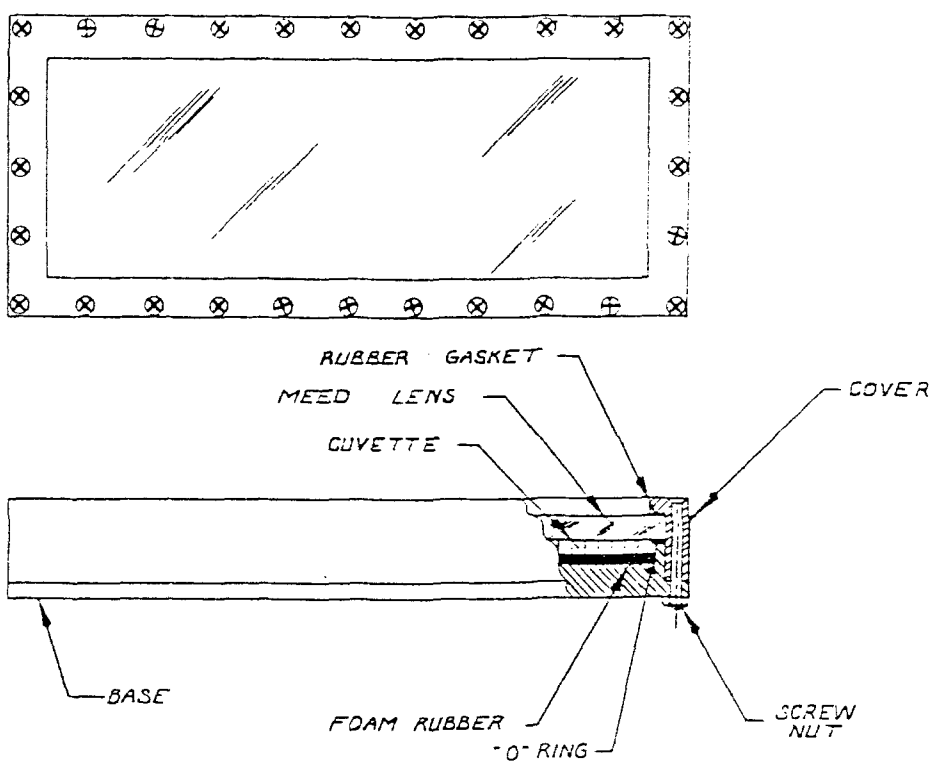

Fig. 3. MEED flight hardware tray construction to hold wet cuvettes unvented to space vacuum. The system maintained a pressure of 14.7 ± 2 psia throughout the space flight experiment. The MEED kept biological samples at temperatures of 20 °C ± 5 °C.

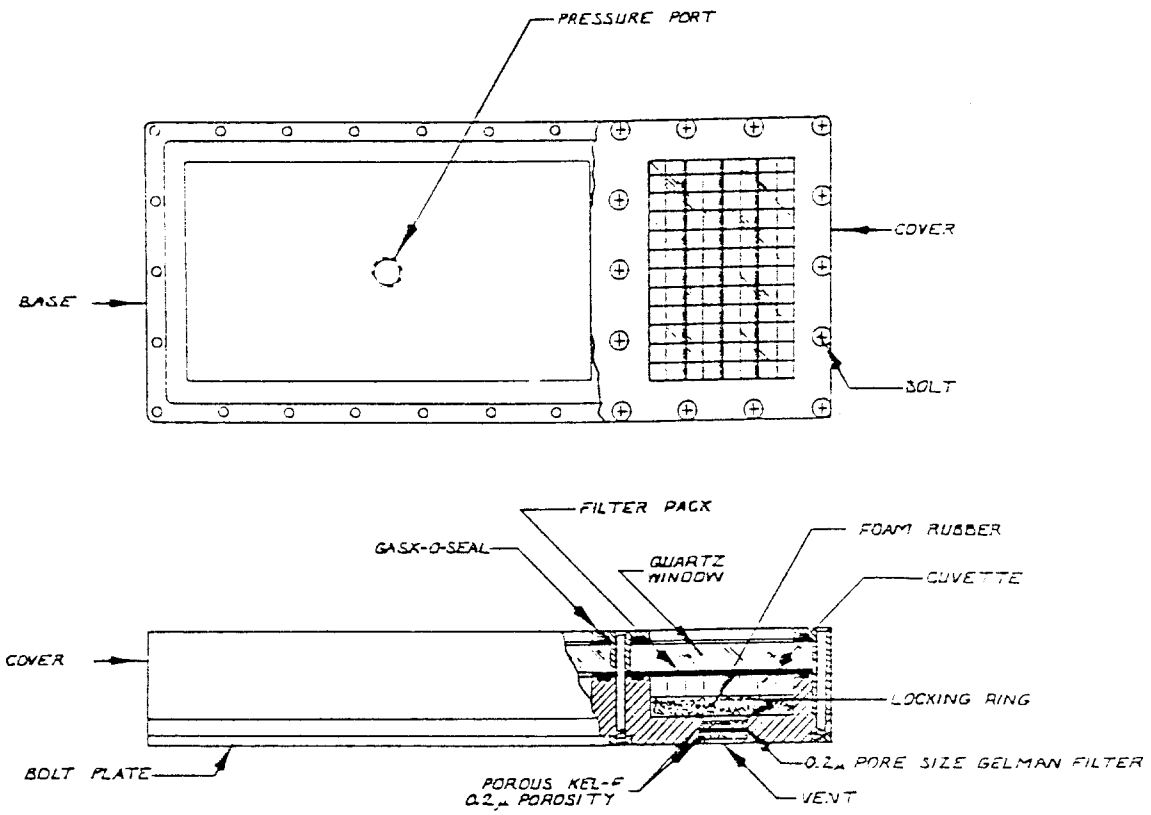

Fig. 4. The MEED flight hardware tray construction with pressure port to allow cells in dry cuvettes to be vented to space vacuum. The MEED held mechanical integrity under shock loads of 78 g's for 25 milliseconds. The filter pack consisted of two filters each. Quartz bandpass interference filters provided peak wavelengths of 254, 280 or 300 nm while quartz neutral density filters regulated the total radiant energy between 4×10^1 ergs cm^2 to full sunlight penetration in space of 8×10^8 ergs cm^2. One each of the two filter types controlled the total radiant energy and peak wavelength entering each cuvette chamber.

Results

Preflight studies, flight preparations and launch procedures of the MEED studies were housed in the Preventive Medicine Division of NASA JSC [46], while postflight studies were conducted at participating NASA centers and at universities. Distribution of the fungal species following the Apollo transearth return flight was conducted for specific examination of the test systems. Detectable changes produced in the living organisms during the space flight exposure were then measured upon return of the MEED to Earth [45, 47]. Medically designed studies directed attention to survival, macroscopic, microscopic, and physiological changes in the space flown fungi [43].

Weightlessness and stress encountered during the launch and splashdown resulted in no variation in viability as indicated in ground control, vibration control, and darkness or flight control in postflight analyses [28]. However, changes in survival rates and phenotype counts, nutritional requirements, and growth rates did vary in relation to space flight parameter exposure levels compared with controls [44].

Rates of phosphate uptake were approximately twice as great for *S. cerevisiae* single cell phenotypic isolates exposed to space parameters as for the wild type ground control [5]. Quantitative determination of ^{32}P was performed by liquid scintillation spectrometry utilizing Cerenkov radiation counting techniques [4]. Alteration of the phospholipid content may involve a change in cell membrane physiology affecting ion transport systems, including the phosphate uptake mechanism [12]. The greatest effect on lipid production and

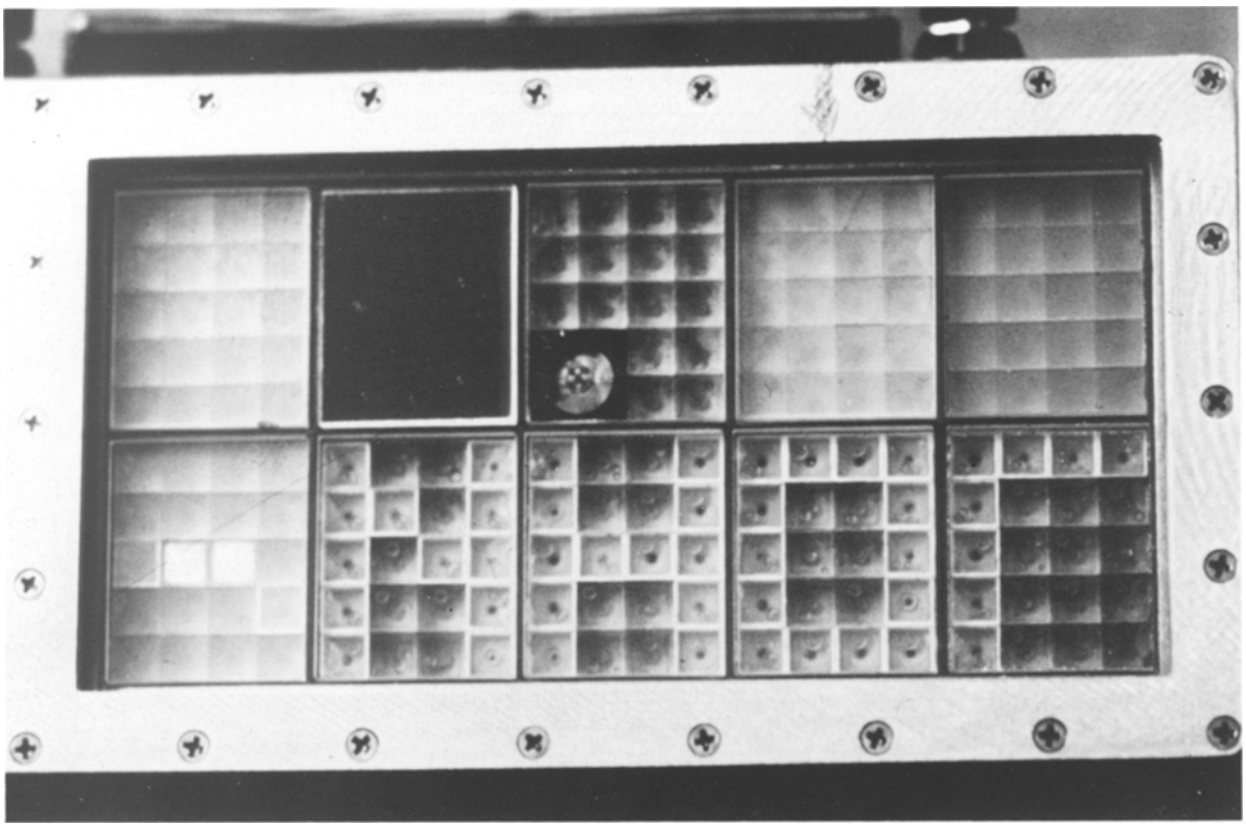

Fig. 5. One flight hardware tray containing compartments of wet and dry type cuvettes. The fill port in wet cuvettes can be easily seen through the quartz exposure windows of each cuvette. Dry cuvettes were constructed to house a monolayer of cells on filter paper adjacent to the underside of the quartz cuvette window.

phosphoglyceride synthesis is radiation received in space at 254 nm in *R. rubra* cells [4].

The synaptinemal complex, chromosome behavior, centriolar bodies and asynchronous disjunction of chromosomes in the meiotic configuration of *C. globosum* were examined with a basic variation pattern found in other Ascomycetes [20, 22]. Diversity in mitotic division proceeded with round compact nuclei, attenuated nuclei and ring or semi-ring nuclear forms in *C. globosum* [23].

Reexposure on Earth with UV radiation showed one postflight isolate to be susceptible to UV reexposure, while other postflight cells were resistant and demonstrated no change in addition to that gained in space [28].

Variations were noted in induced inflammatory response animal lesions among recovered space flight phenotypes as compared to the wild type ground control. Results were similar to data collected immediately after *S. cerevisiae* cell recovery from space, and after a seven year period [27]. Cells exposed to space flight parameters retained a higher recovery rate in dermal lesions compared with cells isolated from lesions induced by wild type controls [16].

Increases in drug assay disk sensitivity were noted with all yeast space flight phenotypes exposed to test parameters of 254, 280, 300 nm UV light and flight control of space flight cuvettes not exposed to light. Test drugs were known to interfere with nucleic acid synthesis, suppress ribosomal activities, and inhibit enzyme production and biosynthesis of proteins [55].

Respiratory deficient yeast cells devoid of cytochromes b, a and a_3 are resistant to light, near UV

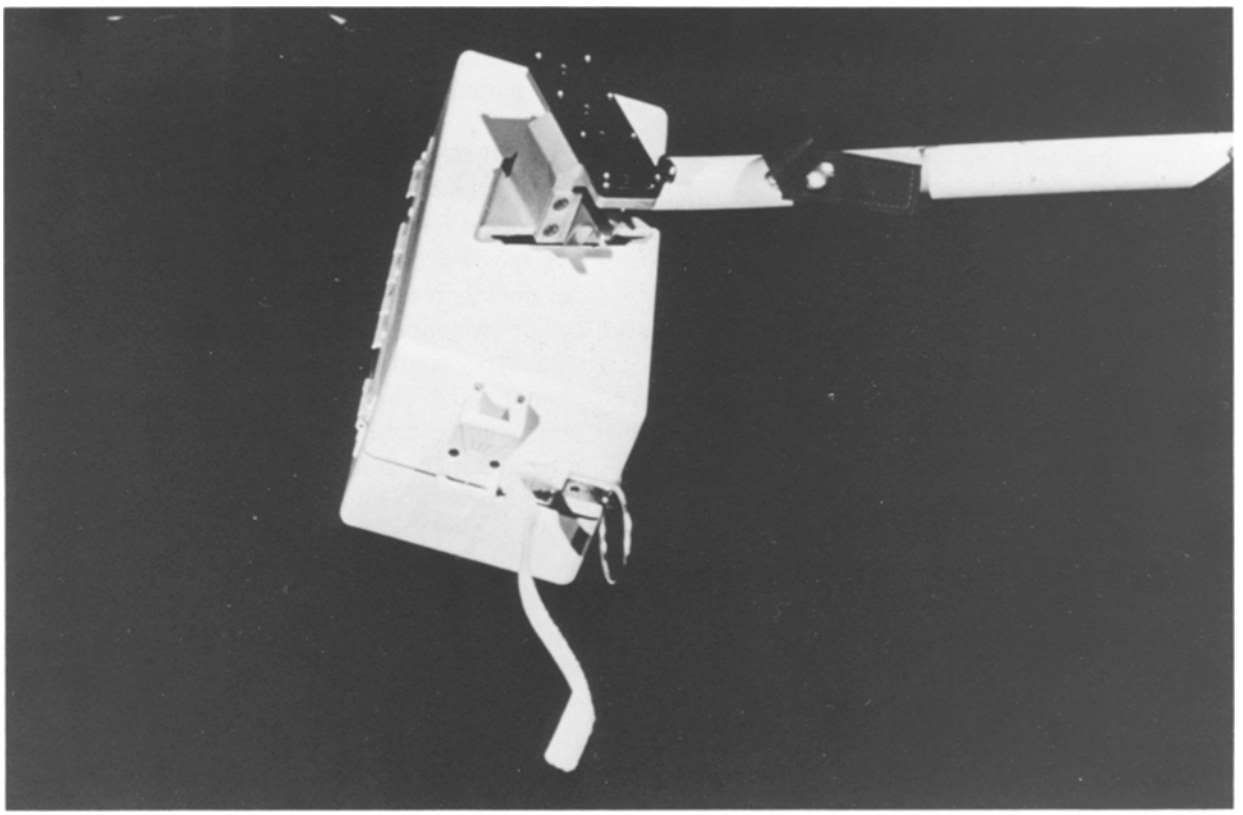

Fig. 6. The MEED flight hardware in the closed position in space. The handling strap, sunsight and image field alignment can be seen on the end side of the hardware. A visual check was made in space for the computerized spacecraft maneuver that allowed a 90° angle deployment toward the sun.

and UV light damage. Mutational events altering yeast cytochrome content may have caused change in growth and respiration rates in *S. cerevisiae* cells exposed to UV radiation in deep space [29].

Increased infectivity and degradation of human hair is apparent with *T. terrestre* flight phenotypes when compared with wild type control at the light and electron microscopy levels [21, 41]. Changes such as the amount and location of conidial development, hyphal development in and on the hair shaft, invaginations along the shaft and colony differences found with space phenotypes when grown on one human hair source were recorded with the *in vitro* studies.

The cellulolytic fungus *C. globosum* lost pigment forming capabilities, caused a reduction of perithecial development, and demonstrated a variation in a-amylase enzyme production when exposed to space flight parameters [17]. Select flight phenotypes exposed to solar irradiation had either no perithecia or immature perithecial development. Flight control isolates produced normal perithecial development. The MEED flight phenotypes exhibited higher a-amylase production compared to ground control.

Phenotype counts for flight cuvettes and survival rates for control cuvettes were each higher in comparison to the remaining cuvettes [53]. Exposure to space flight environments does induce changes in biological systems as identified by the four MEED flight fungi.

Hyphal growth dynamics of *T. terrestre* and *C. globosum* space flight phenotypes such as colony perimeter growth density, protoplasmic leakage of hyphal apices damaged by UV light

irradiation, abnormal growth at the hyphal apex, forked hyphal branches, irregular hyphal walls, and additional morphological changes were attributed to space flight exposure [52]. The nuclear behavior in vegetative hyphae of *T. terrestre* was also examined [19].

Space flight phenotypes and parent isolates of *C. globosum*, *T. terrestre*, *R. rubra*, and *S. cerevisiae* were exposed to pooled salivary samples obtained from healthy individuals and three groups of outpatients. As space flight environmental stress increased in cells housed in the Apollo MEED, fungal growth decreased in the presence of salivary peroxidase activity in saliva from patients receiving radiation treatment for malignancies, protracted corticosteroid regimes for renal complications, and insulin therapy for diabetes mellitus [14]. The peroxidase-mediated antifungal activity in the saliva of normal and host-compromised subjects was used as a test system against the fungal growth of colony phenotypes that were selected and grown from fungal conidia, ascospores and yeast cells. Isolates from control cuvettes were generally more similar to the parent strains, and apparently enzymatic activity studies showed less variation. Space flight phenotypes were more diverse in data analysis.

Each layer of the Apollo and Skylab Extravehicular Modular Unit space suit either directly supported fungal growth, or allowed the diffusion of available nutrients to support fungal growth [49]. *C. globosum* directly degraded and caused deterioration of materials in the multilayered extravehicular activity space suit containing a high carbon content [54]. The pure cotton comfort garment worn beneath the space suit was totally degraded by *C. globosum* enzyme systems when in contact with the fabric layer.

Nuclear weight determinations and reassociation studies on one phenotype and control each of *C. globosum* and *T. terrestre* were made. Genome size of the space flown phenotypes in comparison to the wild type parent strains exhibited increase in both nucleotide pairs per haploid nucleus [26]. The correlation between band sedimentation velocity, homogeneity, and number average molecular weight of the DNA fragments were determined. The size of DNA fragments were determined by sedimentation coefficient methods. The genome size in daltons for *C. globosum* was $2.25 \pm 0.3 \times 10^{10}$ for the wild type and $2.41 \pm 0.16 \times 10^{10}$ for the phenotype, and $1.77 \pm 0.06 \times 10^{10}$ wild type and 2.00×10^{10} phenotype for *T. terrestre*.

Exposure of *T. terrestre* to specific space flight parameters resulted in a phenotype whose whole cell phospholipid contents varied from that of the wild type control [36]. Phosphatidyl inositol, phosphatidyl choline, phosphatidyl serine and phosphatidic acid were found in the wild type, while the space flown phenotype contained phosphatidyl ethanolamine and cardiolipid [37].

A spectrophotometric acid dichromate method for the determination of ethyl alcohol identified variation in alcohol production between phenotypes and control of *S. cerevisiae* [49]. Variation in alcohol production rates appeared to be based on the amount of damage incurred in space by each individual yeast cell.

Space flown phenotypes were morphologically diverse from the parent wild type controls of the four selected fungal species [44]. Hyphal branching patterns, colony development, and cell variation appeared basic to phenotypic change.

Growth rates and changes in fungal phenotypes inoculated on agar containing various carbon and nitrogen sources, and the use of minimal media analysis of vitamin and amino acid requirements indicate variation in space flown phenotypes and wild type controls of the MEED fungi [50]. Cell isolate changes appeared variable in the respective investigations according to the degree of change and damage received by each individual cell while exposed to specific environmental parameters in space.

Summary

The postflight phase of the Apollo MEED mycology attempts to identify survival according to exposure to specific quantitative space flight factors, while the second phase of studies identifies qualitative change other than cell survival

[57]. Initial changes incurred in space on a fungal cell can be monitored and further examined on return of the fungal species test system to Earth. The postflight studies present a better understanding of the space environmental influences on living cells and a more clear understanding of the fungal species under examination.

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