SPACE-RELATED RESEARCH IN MYCOLOGY CONCURRENT WITH THE FIRST DECADE OF MANNED SPACE EXPLORATION

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Abstract. Fungi and Actinomycetes, aside from other microorganisms, have been placed aboard balloons, earth satellites, or high altitude sounding rockets for evaluation of environmental conditions of spaceflight missions. The incursion of harmful effects, including chromosomal breaks or elevated mutation frequencies were identified.

1. Introduction

The purpose of this study involves the accumulation and review of exomycological research. Work discussed on fungi and Actinomycetes primarily was carried out during the first decade of manned space exploration although many studies were on unmanned spacecraft or served as ground base investigations in anticipation of future space flights.

2. The Effects of Orbital Spaceflight Factors on Fungal Species

The U.S. Biosatellite-2 mission was designed as an earth orbiting biological spacecraft. A portion of the unmanned mission contained the *Neurospora* experiment P-1037 in which heterokaryotic cultures of the ascomycete revealed no significant differences in conidial integrity following exposure to earth orbital flight conditions (DeSerres and Webber, 1968a). In preparation for the *Neurospora* experiment, new methods were developed and techniques refined which permitted the fungus to be utilized for space-related studies (DeBusk, 1965). Methods were also refined to insure recovery of the payload (Deliberato, 1965).

The influence of spaceflight factors on genetic and morphological development of Actinomycetes have been described by Glembotskiy *et al.* (1962). Other Soviet workers were not able to demonstrate significant or harmful effects exerted on diploid and haploid yeast cells following exposure to cosmic flight factors aboard the earth satellite Vostok-2 (Air Force Missile Development Centre, 1958a, and Kovyazin *et al.*, 1962a). However, haploid yeast cells previously sensitized with oleic acid were markedly more sensitive to the spaceflight factors than their diploid counterparts (Kovyazin *et al.*, 1962b).

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3. Radiation Effects on In-Flight Fungi

The detrimental effects of radiation on living tissues and microorganisms have been reported (Bender and Stapleton, 1963). Clark (1960) described the influence of heavy particle radiations on *Neurospora crassa* at high altitudes. The studies strongly recommended that heavy shielding equipment be used to protect manned space vehicles against galactic, cosmic, and solar radiations (Clark, 1960 and Grigor'yev *et al.*, 1966). DeSerres (1969) and DeSerres and Webber (1967a), in their presentation of data accumulated from the Biosatellite-2 and Gemini-11 missions, demonstrated that radiation exerted no significant effects on non-dividing spores of *Neurospora crassa.*

The effects of Sr^{85} γ radiation on ground control samples of *Neurospora crassa* in the Biosatellite-A mission were selected as comparative data for future space studies (DeSerres and Webber, 1967b, 1968c). Postflight genetic assays of the experimental payload, however, could not be accomplished since no retrieval was possible (DeSerres and Webber, 1968b).

Jenkins (1968) reported high mutational levels in the fungal spores of *Neurospora crassa* flown aboard the Discoverer-8 and NERV-1 missions. Genetic damage incurred by the ascomycete was confirmed by other workers (Simons, 1958; Simons *et al.*, 1960 and DeBusk, 1961). It was not possible to determine what specific factor of flight contributed to the genetic and physiological changes in the yeast.

Harmful effects exerted on yeast cells following treatment with 660-MeV proton irradiation has been investigated by several Russian researchers (Benevolenskiy *et al.*, 1968). However, experiments conducted with diploid and haploid yeast cells of *Saccharomyces vini*, strain Megri 139-13, and *S. cerevisiae*, strain 40-2587, respectively, showed no significant effects by the proton radiation treatment after comparisons were made with data gathered from studies utilizing γ radiation (Benevolenskiy *et al.*, 1966).

Yeast cells flown aboard the Russian spacecraft, Kosmos-110, promised to be potential sources of proteins, carbonhydrates, and vitamins on spaceflights (Nefedov *et al.*, 1966). Farr (1963) cytochemically studied the cell wall components of *Linderina pennispora* NRRL 2237, and reported the absence of cellulose. The data emphasized the need to identify enzymatic reactions within the microorganisms to allow total assimilation of cell wall substances in the event that they should be selected to serve as foodstuffs in a space program.

4. The Effect of Zero Gravity

Delahaye (1966) reported genetic changes in selected yeasts aboard the Soviet Sputnik and Vostok flights in response to the effects of radiation and weightlessness. Cells of *Neurospora* sp. flown aboard Gemini-3 showed no significant effects between zero gravity and radiation (Jenkins, 1968), but a 2-fold increase in the number of chromosomal breaks was confirmed at all radiation levels after exposure to P^{32} β radiation.

The NASA Biosatellite Program (Dallow *et al.*, 1966; Jenkins, 1964 and Thimann,

1968) has been actively involved in the study of microorganisms exposed to the space environment, and much effort has been directed towards a clarification and re-evaluation of the effects of weightlessness and radiation. The Gemini-11 mission indicated that no significant synergistic effects of radiation and zero gravity were exerted on asexual spores of a 2-component heterokaryon (DeSerres *et al.*, 1969 and DeSerres, 1968). Bender (1967) and Bender *et al.* (1967a and b) presented comparable data from the Gemini-11 S-4 radiation spaceflight interaction experiments confirming earlier investigations of possible synergistic effects of weightlessness and radiation.

DeSerres and Webber (1968a) reported that ionizing radiations in the form of cosmic rays, trapped particles in the Van Allen Belt, or proton fluxes associated with flares posed severe limitations on the screening techniques used to select microorganisms for orbital flight studies. Investigations have shown that radiation exposure to astronauts should be the major concern of spaceflights on extended missions (Tobias *et al.*, 1965). The accumulation of very significant levels of proton and alpha particle radiations in the outer body layers were reported.

5. Viability Studies of Fungal Species Exposed to Orbital Flight Missions

Fungal cells of *Penicillium roqueforti* Thom were highly resistant to drying and prolonged storage aboard the Agena mission (Hotchin, Lorenz, Markusen and Hemenway, 1967). The Gemini-9-A earth satellite confirmed the survivability of *P. roqueforti* Thom following direct response to the space environment for 6.5 and 17 hr (Hotchin *et al.*, 1967). Viability of the fungi for 6 hr was reported in daylight approaching an altitude of 34 km, the limits of the terrestrial atmosphere (Hemenway *et al.*, 1965). At an altitude of 160 km, spore viability lasted for 3 min, the longest exposure period possible using sounding rockets. The results were of significance in that they contradicted previously held predictions that immediate sterilizations of the terrestrial microbes would occur during periods of direct exposure to the space environment.

Similar viability results for Actinomycetes were reported by Glembotskiy *et al.* 1962). In these studies, *Actinomyces erythreus*, strains 2577 and 8594, were flown aboard the second Soviet satellite-spaceship. *Actinomyces aureofaciens*, strain LS-B-2201, was flown aboard a fourth mission. While viability was significantly reduced for strain 8594 of *A. erythreus* and *A. aureofaciens*, a 6-fold enhancement in survival of *A. erythreus*, strain 3577, was reported. Gyurdzhian (1962) presented evidence for positive and negative deviations in the viability of 4 actinomycetous strains aboard spacecraft.

The viability of terrestrial microorganisms flown aboard balloons, rockets (Hemenway *et al.*, 1965, 1966), and Gemini satellites (Hemenway *et al.*, 1967) are documented. The lethal effects of solar radiations are discussed, and interpreted in light of the survivability of space-flown microorganisms. Lorenz *et al.* (1968) reported results from the Gemini-11-A, Gemini-12, and Agena-8 satellite-borne exposure and collection experiments revealing the absence of viable microorganisms on sterile, methylcellulose collection surfaces. Kvostova *et al.* (1962), however, showed spore viability to

be significantly increased in the *uv*-sensitive strain 8594 of *Actinomyces erythreus* flown on board the second Russian spaceship. Reports of mycelial growth and proliferation for strains 2577 and 8594 of *A. erythreus* were presented in the same series of experiments thus confirming earlier reports (Glembotskiy *et al.*, 1962). Kovyazin *et al.* (1962a), in summarizing results of experiments in which suspensions of haploid and diploid yeast cells were exposed to 25 hr of cosmic flight factors following sensitization with oleic acid, reported that no significant effects were exerted on the viability of in-flight microorganisms. The sensitized haploid yeast cells, however, displayed relatively greater viability decreases when compared to the diploid organisms.

6. The Effects of Simulated Extra-Terrestrial Environments on Fungal Microorganisms

Viability and survival studies of fungi subjected to simulated extra-terrestrial environments have been examined with positive results (Benner, *et al.* 1963a, and Silverman *et al.*, 1963). Zhukova and Kozlova (1966) 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 (Cameron *et al.*, 1970). 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 (Siegel *et al.*, 1963). In other experiments, Mardres (1966) determined the viabilities of selected microorganisms following their exposure to elevated *uv* flux similar to conditions on the Martian surface.

The ability of a Martian environment to support growth of unicellular and multicellular organisms has been reported (Hubbard and Miller, 1967; Curtis, 1967; Kozlova and Zhukova; 1966 and Lozina-Lozinskiy, 1962). 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 (Benninghoff, 1964 and Lozina-Lozinskiy, 1962).

The ability of fungal spores to withstand ultrahigh vacuum was confirmed by several workers (Agadzhanyan *et al.*, 1965; Bueker, 1966 and Silverman *et al.*, 1967). A high degree of resistance was noted for aerobic, spore-bearing fungi subjected to 10^{-6} torr. *Aspergillus niger* was among the most highly resistant fungi following combined treatment with ultrahigh vacuum and *uv* light.

Cameron *et al.* (1970) demonstrated no survival for mold cells after 5 yr of continuous exposure to ultrahigh vacuum conditions in the range of 10^{-7} to 10^{-8} torr. Species of *Aspergillus* and *Penicillium* did not survive 10^{-9} torr for 30 days (Brueschke *et al.*, 1961) while *A. fumigatus* survived 10^{-16} torr for 5 days (Portner *et al.*, 1961). The data suggested that other environmental parameters deserved further study in

order to clarify the nature of harsh, terrestrial environments and the possibility of life on other planets.

Berliner and Neurath (1964) subjected luminescent and dikaryotic cultures of *Panus stypticus* to uniform magnetic fields of 1100 Oe cm^{-1}, and noted that the effects on colonial morphology and total light output were not significant. Moskwa and Rostkowska (1965), however, reported that magnetic fields of 2400 to 3200 Oe cm^{-1} exerted decrimental effects on CO_2 production in yeast cells. The accumulation of toxic substances, including atabrine, methane, and dihydrochloric acid was verified. Sale and Hamilton (1967) confirmed earlier reports on the lethal effects of high electric fields.

7. The Control of Space-Flown Fungal Contaminants

The problem of contamination in spacecraft compartments has been examined. Portner (1965) described the nature of microbial contamination within irradiated and heated electronic components, while other workers (Albrecht *et al.*, 1963 and Leonard and Klemme, 1962) have described techniques for controlling contamination of jet hydrocarbon fuels by microbial organisms. Mayumi (1968a, b) reported that the 'kerosene fungus', *Cladosporium resinae*, was a common fungal species isolated from jet fuel systems. Aluminum corrosion by the fungus was shown to be very extensive. Sludge deposits on the panelling units of the integral tank of a YS-11 jet aircraft were demonstrated. Lizuki *et al.* (1966) presented methods for the isolation and purification of toxic substances in *Candida rugosa*, strain JF 101 from aircraft fuel. Evidence for the diterminal oxidation of *n*-decane by the fungus was presented. Methods have been described which promised to reduce aircraft fuel contamination without the use of biocidal additives (Hazzard, 1963). Presently, the best solution to the problem of fungal fuel contamination lies in the recognition of the contamination source, and the subsequent enforcement of good 'house-keeping' techniques (Leonard and Klemme, 1962).

Wilkins (1969) noted that the following fungi proliferated in the air, H_2O, and table-top surfaces aboard a manned ground-based integrated life support system: *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp., and *Pullularia* sp. Other fungal contaminants included *Acrothecium* sp., *Stemphylium* sp., *Dendrostilbella* sp., *Helminthosporium* sp., *Monosporium* sp., and *Monotospora* sp., as well as a variety of yeasts. Integrated crew and command module microbial investigations of preflight and postflight NASA Apollo missions currently examine the microbial load and fungal species of Apollo spaceflights.

The sonochemical approach in controlling contamination is a new inception (Pisano, 1967). Its effectiveness was believed by Burns (1967) to lie in the significant decrease in protein synthesis initiated by sub-lethal doses of sonic irradiation treatments, and in the drastic permeability alterations incurred by the cell membrane. Hughes and Neppira (1964) reported a complete disintegration of yeast cells following treatment with high-intensity ultra-sound, whereas marked changes in yeast RNA solutions were noted by other investigators (Levinson and Nefedov, 1964). Control of

contamination with high-intensity sound waves has gained popularity in industry but the method is not extensively used in space studies.

Yeasts, filamentous fungi, and Actinomycetes are spacecraft contaminants, and they are the subjects of defined space experiments. Space irradiations and temperature extremes have had extensive mycological studies which will serve as ground control information for future space-related research, however, these areas were not extensively searched for this review.

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