# INDUCED LESIONS IN MICE CAUSED BY SACCHAROMYCES CEREVISIAE WILD-TYPE AND SELECTED PHENOTYPES OF THE APOLLO 16 MICROBIAL ECOLOGY EVALUATION DEVICE

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Abstract: Saccharomyces cerevisiae Hansen was exposed to spaceflight parameters during the deployment of the Microbial Ecology Evaluation Device on Apollo 16, then returned to earth for postflight analysis. Space parameters included ultraviolet light at known wavelengths and intensities in addition to weightlessness. Induced lesions caused by inoculations of the spaceflight cells were systematically monitored in mice. Variations occurred among the recovered phenotypes as compared to the wild-type ground control. Results were similar to data collected immediately after yeast cell recovery from space, after a seven year period of colony growth and transfer.

Materials and Methods: Vegetative yeast cells of Saccharomyces cerevisiae Hansen y2439 were housed in distilled water or dry in 0.05 ml volume square cuvettes within the Microbial Ecology Evaluation Device (MEED) spaceflight hardware of Apollo 16. Each cuvette contained a quartz window and a series of filters to regulate the ultraviolet light (UV) wavelength and intensity at exposure in space (Taylor, 1970). The MEED was deployed at a 90° angle to the sun for 10 min + 7 sec during the transearth Extra Vehicular Activity (EVA) of Apollo 16 (Volz, 1975). Fungi in the flight hardware were exposed to 254, 280, and 300 nanometers (nm) UV light at various energy levels during deployment and attachment of the MEED flight hardware on the television campole extension and Command Module hatch (Volz et al., 1974). After exposure, the flight hardware was stowed and returned to the laboratory at splashdown (Volz, 1974). Fungal cells housed in the flight hardware were placed on Sabouraud maltose agar and initially studied for survival capabilities according to exposure levels in space (Volz and Dublin, 1973).

Phenotypes for the present study were obtained from viable cells collected in postflight analysis. The phenotypes were selected by alterations in colony morphology and growth rate (Volz, 1973). Six phenotypes and the wild-type ground control were used in the current study. The seven isolates were maintained for seven years in continual growth and colony transfer on Sabouraud maltose agar.

Swiss Flow DUB/KR mice were pretreated with 20 mg/ml inoculations of hydrocortisone succinate, two inoculations at one week intervals, for two weeks prior to the introduction of the yeast test organisms to repress the defense system of the animals.

Mice then received 0.2 ml cell suspensions in 0.9% saline intraperitoneally, at a cell concentration of $1 \times 10^9$ cells per ml, with three inoculations at one week intervals. Autopsy was performed

when changes in the normal behavioral activity in the animals were noted, two weeks after inoculation. Approximately 0.25 g material from isolated lesions were inoculated on Sabouraud maltose agar for yeast cell recovery. Replicates of three animals were used for each test phenotype and control.

Results: The spaceflight exposures received by the phenotypes are presented in Table 1. Morphological diversification in phenotypes compared to the parent strain, survival rates and exposure parameters were the principal method in selection of the test organisms for the present study.

Viable cells were recovered from mouse tissue streaked on agar plates as shown in Table 2. Subcutaneous mouse lesions of S. cerevisiae wild-type and phenotypes were large and quite well circumscribed. Yeast cells were recovered from the subcutaneous region, however, the cells did not involve the overlying skin. Budding yeast cells were very abundant. Liver lesions initiated by phenotype 7012-4 were large, while lesions caused by phenotype 7027-2 were small and fairly walled off. These results are similar to those of Hiebel and Volz (1977) who found that the lesions were necrotic and infiltrated with a mixture of polymorphonuclear cells surrounded by monocytes, including many lymphocytes and macrophages.

Discussion: Varying degrees of inflammatory responses were noted with the wild-type and phenotypes. Order of reactivity observed in mice from the most severe to the least was phenotype 1420-1, 7027-2, 7024-2, 1435-2, 1440-1, the parent strain, and phenotype 7012-4. Most of the phenotypes gave very diffuse and very intense reactions sometimes to the point of being a true abcess, and many of the cells would often proliferate in the host.

Variations in host reactions were noted between the yeast phenotypes that were initially exposed to specific wavelengths and intensities of UV light in space. In all cases but one, more viable cells were recovered from the dermal lesions induced by the phenotypes than from the lesions initiated by the wild-type ground control.

Of the four fungal species included in the Apollo MEED experiments, S. cerevisiae phenotypes produced the most severe inflammatory responses at yeast phenotype recovery immediately after exposure to the space environment (Volz and Hiebel, 1977). After seven years of continual growth in the laboratory, the S. cerevisiae isolates in the current study demonstrated similar severity in producing induced lesions in Swiss Flow DUB/KR mice.

Summary: The cellular response to Saccharomyces cerevisiae Hansen and spaceflight phenotypes was a foreign reaction and induced lesions. Response variation was a result of the exposure parameters to UV light. Phenotypes, with the exception of one, retained a higher recovery rate in lesions compared with cells isolated from lesions induced by the wild-type.

## References

Hiebel, T. L. and P. A. Volz. 1977. Foreign body reactions induced by fungi irradiated in space. Phytologia. 35:365-372.

Taylor, A. M. 1970. Final report design feasibility study for construction of a Microbial Ecology Evaluation Device (MEED). NAS 9-10830. Aerojet Medical and Biological Systems, El Monte, California. 186 pp.

Volz, P. A. 1975. Apollo 16 MEED Mycology. Phytologia. 31:193-225.

Volz, P. A. 1974. The Apollo 16 Microbial Ecology Evaluation Device mycology studies. NAS 9-11562. National Aeronautics and Space Administration. Houston, Texas. 325 pp.

Volz, P. A. 1973. Mycological studies housed in the Apollo 16 Microbial Ecology Evaluation Device. In: Proceedings of the Microbial Response in Space Environment Symposium. pp. 121-145.

Volz, P. A. and M. Dublin. 1973. Filamentous fungi exposed to space-flight stresses including known levels of ultraviolet irradiations. Space Life Sciences. 4:402-414.

Volz, P. A., Y. C. Hsu, J. L. Hiser, J. M. Veselenak, and D. E. Jerger. 1974. The Microbial Ecology Evaluation Device mycology spaceflight studies of Apollo 16. Mycopathologia et Mycologia Applicata. 54:221-233.

Table 1. UV light exposure parameters for the yeast strains selected for induced lesion studies.

| Phenotype | Wavelength (nm) | Intensity (ergs/cuvette/ 10 min) | Cuvette Moisture |
|---|---|---|---|
| Parent[@] | --- | --- | --- |
| 1420-1 | 280 | $2.4 \times 10^4$ | wet |
| 1435-2 | 300 | $7.0 \times 10^4$ | wet |
| 1440-2 | 254 | $3.5 \times 10^4$ | wet |
| 7012-4 | 280 | $9.4 \times 10^3$ | dry |
| 7024-2 | 300 | $4.2 \times 10^4$ | dry |
| 7027-2 | 254 | $9.6 \times 10^3$ | dry |

[@]Parent wild-type control culture housed on ground at 25 C with no light exposure.

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Table 2. Average number of colony forming units per plate obtained from wild-type and phenotypes recovered from mouse tissue.

| Strain | Skin | Liver | Kidney | Spleen |
|---|---|---|---|---|
| Parent | 9 | 6 | 2 | 9 |
| 1420-1 | 490 | 0 | 0 | 0 |
| 1435-2 | 111 | 0 | 0 | 0 |
| 1440-2 | 51 | 0 | 0 | 0 |
| 7012-4 | 6 | 5 | 0 | 0 |
| 7024-2 | 170 | 0 | 1 | 0 |
| 7027-2 | 303 | 1 | 0 | 0 |