DNA ANALYSIS OF FUNGAL WILD TYPES

AND SPACEFLIGHT PHENOTYPES

Douglas E. Jerger and Paul A. Volz
Mycology Laboratory, Eastern Michigan University
Ypsilanti, Michigan 48197

Abstract: Ascospores of Chaetomium globosum and conidia of Trichophyton terrestre were exposed to ultraviolet light in space on board the flight of Apollo 16. Genome size of the space flown phenotype appeared larger than the wild type of each species based on the number of samples examined.

Introduction: Two wild type filamentous fungi, Chaetomium glosobum ATCC 6205 (Hsu et al., 1973a, 1973b) and Trichophyton terrestre CDC x285 (Hsu et al., 1974) were included for study in the Apollo 16 Microbial Ecology Evaluation Device (MEED) (Volz, 1975). During the transearth Extra Vehicular Activity (EVA), the MEED was deployed at a sun synchronous 90^{o} angle for 10 minutes 7 seconds to specific spaceflight parameters of ultraviolet light and energy levels according to the spaceflight hardware design (Taylor, 1970). Ground control and spaceflight ascospores and conidia were housed dry or in sterile distilled water in the cuvette hardware (Volz, 1974). After the exposure to space parameters the ascospores and conidia were returned to the laboratory in the MEED hardware and allowed to germinate for postflight evaluations (Volz et al., 1974).

Materials and Methods: One phenotype of each species in addition to the parent or ground control strain was selected for base ratio comparison and genome size evaluations. Phenotype T. terrestre 7085-4 was isolated from the spaceflight cuvette receiving light irradiation of 300 nm at 4.2×10^{4} ergs, while the C. globosum 6904-1 phenotype was exposed to full direct sunlight in space at 1.5×10^{7} ergs. The phenotype isolates were morphologically diverse from the parent wild types (Volz, 1973).

The isolates were maintained in culture tubes on Sabouraud's maltose agar and allowed to sporulate. The spores were harvested by washing and centrifugation, inoculated into one liter of Sabouraud's maltose broth in two liter Erlenmeyer flasks and shaken at room temperature for 72 hours. Mycelium was harvested and ground in liquid nitrogen which yielded DNA preparations with a high molecular weight suitable for analysis by buoyant density centrifugation and band velocity sedimentation (Storck and Alexopoulos, 1970).

DNA extraction and purification followed similar techniques previously described by Marmur (1961) and Villa and Storck (1968). The DNA was obtained from the nuclei by cellular disruption, centrifugation, deproteinization, RNase treatment, phenol extraction

and ethanol precipitation. The base composition of the DNA was determined from its buoyant density in CsCl (Meselson et al., 1957; Sawyer et al., 1975; Schildkraut et al., 1962). This method demonstrates a linear relationship between buoyant density and guanine - cytosine content of the DNA. All buoyant densities were related to that of Mucor bacilliformis.

The DNA samples were sheared in a French pressure cell, precipitated, redissolved, layered on a hydroxyapatite column for purification and dialyzed for molecular weight determinations (Dutta and Ojha, 1972). Procedures reported by Studier (1965) were used to determine the correlation between band sedimentation velocity, homogeneity, and number average molecular weight of the DNA fragments. The size of DNA fragments were determined by sedimentation coefficient methods (Studier, 1965). The molecular weights and average number of nucleotides per single stranded DNA fragment for the test organisms were: Chaetomium globosum, 1.19×10^5 and 236; Trichophyton terrestre, 1.05×10^5 and 324; Bacillus subtilis, 1.11×10^5 and 334. The Bacillus subtilis genome was used as the control estimate for accuracy comparison to the test species. Genome size of the test isolates was estimated from the proportionality relationship between the $Cot_{0.5}$ of the unknown (Britton and Kohne, 1968). The molecular weight standard used for B. subtilis was 2.0×10^9 daltons and the $Cot_{0.5}$ value was 0.7.

Results: Guanine - cytosine contents were calculated on the preflight control organisms to compare with previously reported data as well as with postflight phenotype results. Chaetomium globosum 6205, run with M. bacilliformis as the standard, had a calculated % GC of 51 as previously reported by Storck and Alexopoulos (1970) while Trichophyton terrestre 285 also exhibited a 51% GC base ratio which falls within the range reported for Deuteromycetes. Following spaceflight exposure, the %GC determinations were calculated for one phenotype each of C. globosum and T. terrestre. No significant differences in the GC base ratio were found between the phenotype and the parent.

Haploid genome DNA content determinations and reassociation studies were performed on one phenotype each of C. globosum and T. terrestre (Table 1). The melting temperature determining 50% of the total hyperchromicity of single stranded DNA fragments included T. terrestre wild type, 72.5° C and T. terrestre phenotype, 72.0° C 1/10 SSC (0.015 M NaCl + 0.0015 M trisodium citrate, pH 7.0); and C. globosum wild type, 90.7° C and C. globosum phenotype, 89.5° C 1 x SSC (1.5 M NaCl + 0.15 M trisodium citrate, pH 7.0). The reassociation of a pair of complementary sequences of the DNA of each organism was characterized by the value of Cot as shown in Fig. 1. The mean value of the separate experiments is presented in the data. Major repetitive DNA components of the control and test organisms were not detectable following the experimental technique of the second order rate plot for the renaturation of DNA (Wetmur

and Davidson, 1968). The $Cot_{0.5}$ values for the organisms were C. globosum wild type, 8; C. globosum phenotype, 8.6; T. terrestre wild type, 6.4; and T. terrestre phenotype, 7.1.

Discussion: The selection of the fungal species for the MEED spaceflight hardware was carried out in preflight studies at the NASA Johnson Space Center, Preventive Medicine Division. Criteria for species selection included the ability of the organism to survive constraints of the spaceflight hardware, the usefulness of the species for identifying change in the microorganisms incurred in space, previous space research in mycology related to the proposed studies, and ease of handling the organisms (Dublin and Volz, 1973). Preflight tests on numerous species representing most all major classes of fungi also assisted in selection of the flight organisms (Volz, 1974).

Chaetomium ascospores and Trichophyton conidia exposed to specific ultraviolet light irradiations in space of known wavelengths and intensities demonstrated variable survival rates and phenotype counts (Volz et al., 1974). Variations in hyphal morphology were also attributed to exposure to irradiation (Volz and Dublin, 1973). Significant variations occurred in fungal growth rates according to phenotype association with specific salivary samples of healthy individuals as control and to saliva from patients receiving radiation treatment for malignancies, protracted corticosteroid regimes for renal complications, and insulin therapy for diabetes mellitus. Host compromised saliva was less able to reduce growth rates of test fungi compared with normal saliva. As spaceflight environmental stress increased in irradiated cells, fungal growth decreased in the presence of salivary peroxidase activity.

Other studies indicate variation in space flown phenotypes from that of the wild type or ground control. A space flown phenotype of T. terrestre compared with the wild type differed in phospholipid content (Sawyer, 1975). Exposure of T. terrestre to specific spaceflight parameters resulted in a phenotype whose whole cell phospholipid content varied from that of the wild type. Preliminary results in other studies including nutritional requirements, exposure to antifungal drugs, induced lesions in mice and hamsters, and cell metabolites indicate change when characterizing the parent strain with phenotype cell isolates obtained from space flown cuvettes (Volz, 1975). In the current study phenotypes exhibiting diverse morphological variation from the parent wild type strains were selected for nuclear weight determinations and reassociation studies. Slight increases in genome size comparisons were observed, however, the differences fall within the experimental error of the analytical techniques.

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Table 1.

Genome size comparisons of the wild type and space flown phenotype (mean ± standard deviation)

Chaetomium globosum

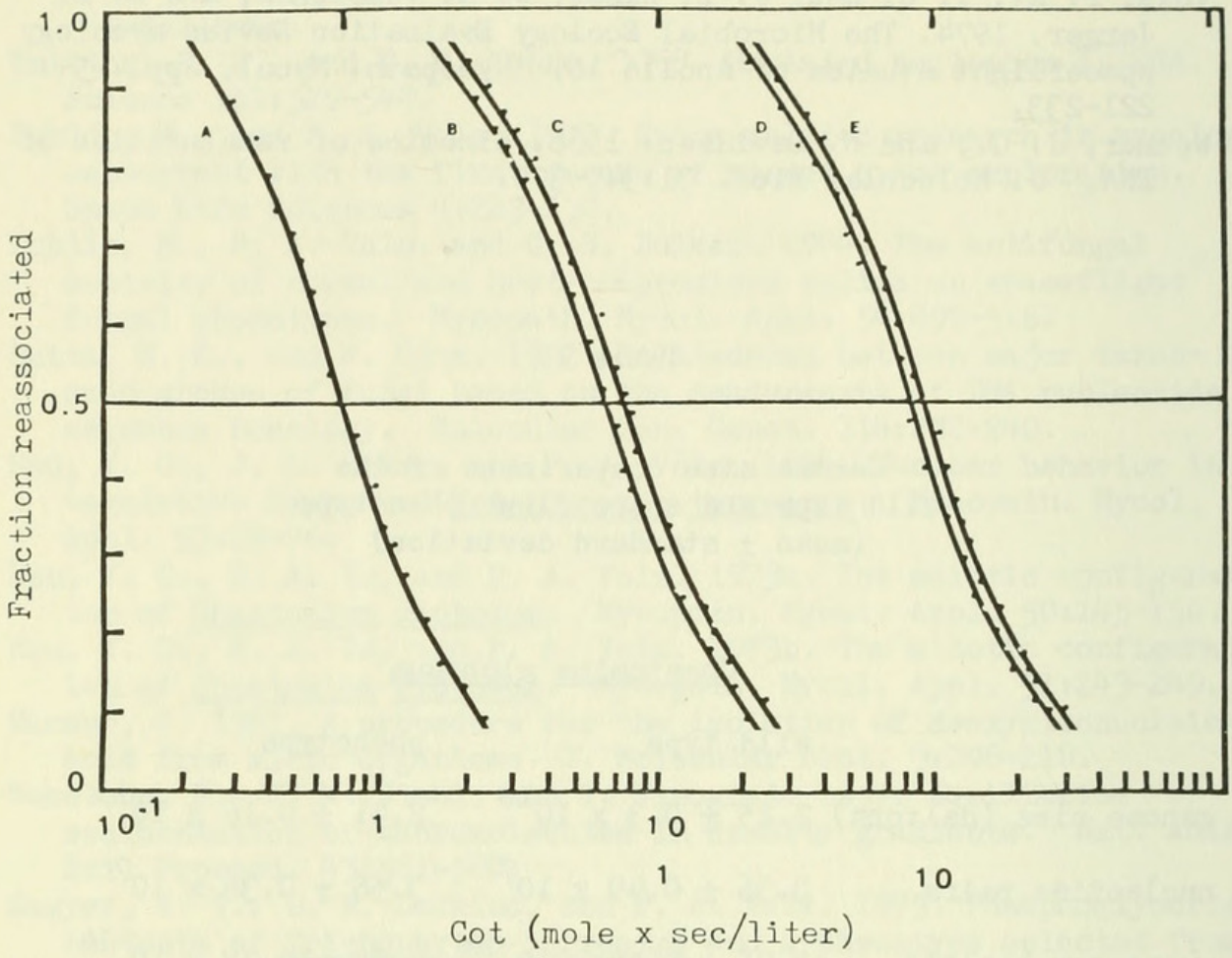
	wild type	phenotype
genome size (daltons)	$2.25 \pm 0.3 \times 10^{10}$	$2.41 \pm 0.16 \times 10^{10}$
nucleotide pairs	$3.36 \pm 0.49 \times 10^{7}$	$3.56 \pm 0.36 \times 10^{7}$
grams per haploid nucleus	$3.75 \pm 0.57 \times 10^{-14}$	$4.00 \pm 0.28 \times 10^{-14}$

Trichophyton terrestre

	wild type	phenotype*
genome size (daltons)	$1.77 \pm 0.06 \times 10^{10}$	2.00×10^{10}
nucleotide pairs	$2.57 \pm 0.12 \times 10^{7}$	3.05×10^{7}
grams per haploid nucleus	$2.93 \pm 0.16 \times 10^{-14}$	3.3×10^{-14}

*T. terrestre phenotype reassociation was only performed on one sample.

Figure 1.



Reassociation of double-stranded nucleic acids from A. B. subtilis, B. T. terrestre wild type, C. T. terrestre phenotype, D. C. globosum wild type, and E. C. globosum phenotype. Log 10 was duplicated to accommodate data.