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Phosphate Uptake in *Saccharomyces cerevisiae* Hansen Wild Type and Phenotypes Exposed to Space Flight Irradiation

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Rates of phosphate uptake were approximately twice as great for *Saccharomyces cerevisiae* single-cell phenotypic isolates exposed to space parameters as for the wild-type ground control. Quantitative determination of ^{32}P was performed by liquid scintillation spectrometry utilizing Cerenkov radiation counting techniques.

Wild-type *Saccharomyces cerevisiae* Hansen ATCC y2439 yeast cells housed in the Apollo Microbial Ecology Evaluation Device (MEED) were exposed at a 90° angle to the sun for 10 min plus 7 s in space during the transearth Extra Vehicular Activity of Apollo 16 (9). Cuvettes were designed to hold either a dry inoculum or a distilled-water suspension of yeast cells. Each cuvette had a quartz window, band-pass filter, and neutral density filter for exposure to specific ultraviolet (UV) light components of solar irradiation (8). Yeast cells were exposed to 254-, 280-, and 300-nm UV light at various energy levels, full solar light, and no light (11). Some yeast cells housed in dry cuvettes were vented to the vacuum of space. Additional controls for the wild-type cells included ground control cells housed at room temperature and flight control cells housed in the flight hardware with no light exposure. At splashdown of Apollo 16, the MEED flight hardware was returned to the laboratory. Space-exposed phenotypes were isolated and maintained on Sabouraud dextrose agar (9, 10). *S. cerevisiae* single-cell phenotypic colonies 7027-2, 7024-2, and 7012-4, isolated from the MEED cuvettes, received 254 nm at 9.6×10^3 ergs of total energy, 300 nm at 4.2×10^4 ergs, and 280 nm at 9.4×10^3 ergs, respectively. 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. Survival rates did vary in relation to space flight parameter exposure levels compared with controls (9). Selected space flight phenotypes were thus examined for ^{32}P evaluations in comparison with the wild type.

Stock cultures of *S. cerevisiae* wild type and phenotypes were maintained on Sabouraud dextrose agar slants at 25°C. Glassware, including the VirTis fermentor vessel, was cleaned with a chromic acid solution to eliminate trace phosphate contamination. Several distilled-water rinses followed, and glassware was autoclaved before use. Two 500-ml Erlenmeyer flasks, each containing 100 ml of sterile dextrose broth, were inoculated with yeast from stock culture tubes. The cultures which served as the fermentor inoculum were incubated on a reciprocating shaker at 183 rpm for 3 days. The inoculum and 6 liters of sterile dextrose broth composed of 40 g of dextrose and 10 g of neopeptone per 100 ml of distilled water were added to the VirTis fermentor model 40-100-A. Aeration was achieved by a Seitz filter (grade S3 size L6, Fisher Scientific Co.) attached to the air entry port of the fermentor. The temperature was maintained at 24°C for 4 days of incubation.

Yeast cells were examined at selected intervals during growth for fungal or bacterial contamination. After incubation, yeast cells were collected in sterile 200-ml centrifuge bottles in an IEC International centrifuge, model PR-2, at 290 $\times$ *g* for 7 to 8 min. The yeast cells were resuspended, washed several times with sterile distilled water, and then placed in the fermentor vessel in sterile 5% (wt/vol) glucose solution. After 24 h of incubation the cells were harvested by centrifugation. Collected cells were placed in the fermentor in 6 liters of sterile distilled water for a 3-day starvation period. Cells were collected by centrifugation. All phosphate uptake experiments were conducted at 25°C under aerobic conditions. Yeast cells (30 ± 0.01 g, wet weight) were added to a 400-ml polyethylene beaker along with 45 ml of 0.55 M glucose solution in 0.1 M succinic acid, adjusted to pH 6.5 by the addition of tris(hydroxymethyl)aminomethane while constantly being mixed. The total volume of yeast suspension was then measured. One milliliter of yeast cell suspension was withdrawn for protein determination performed by a modification of the Lowry method (5). Five milliliters of 8.8 μM phosphate solution

(KH_2PO_4) containing 5 μCi of [^{32}P]phosphoric acid was added to the reaction vessel. The addition of the phosphate to the yeast suspension was designated as zero time for all subsequent measurements. After a 1-min mixing period, 2 ml of yeast suspension at 45.5 ± 5 mg/ml of protein was withdrawn from the reaction vessel by pipette and delivered to a 50-ml polyethylene centrifuge tube containing 3 ml of 4% trichloroacetic acid solution. The mixture was centrifuged immediately in a Sorvall superspeed centrifuge (type SS-1) at 2,300 × *g* for 5 min. One milliliter of the supernatant was carefully pipetted from the centrifuge tube and delivered to a polyethylene scintillation counting vial containing 14 ml of distilled water. Samples (2-ml) were withdrawn from the reaction vessel at 15-min intervals and treated in the same fashion as the first 2-ml sample. The counting vials were placed in a Searle Analytic Delta 300, 6890 liquid scintillation spectrometry system for Cerenkov counting of ^{32}P activity. Background was measured with a counting vial containing 15 ml of distilled water. The window setting was 0 to 5 V.

The phosphate uptake experiment involving a representative of *S. cerevisiae* wild type run in triplicate and three phenotypes as shown in Fig. 1 illustrates the counts per minute of [^{32}P]phosphoric acid remaining in the supernatant at each sample withdrawal period. Average rates of phosphate uptake for the wild type and phenotypes are shown in Table 1. Data required to determine the uptake average rates and the phenotype exposure parameters received in space are also given.

Average rates of phosphate uptake were determined in all experiments by relating the first sample activity measurement in counts per minute, representing 44 nmol of phosphate, with the time it takes for the organism to expend its capability for phosphate uptake represented by the sample with the lowest activity count, representing *N* number of nanomoles of phosphate. Phenotype 7027-2 (254-nm exposure in space) differed the greatest in phosphate uptake compared with the control. The UV light wavelength, rather than the energy dose, appeared to be the more important factor in determining the degree of stimulation of phosphate uptake. Phenotype 7027-2, receiving 9.6 × 10^3 ergs per cuvette per 10 min, was exposed to approximately the same energy level as phenotype 7012-4 (9.4 × 10^3 ergs per cuvette per 10 min); yet phenotype 7027-2 had a higher average rate of phosphate uptake (0.38 nmol of phosphate per min per g of yeast protein) than did phenotype 7012-4 (0.27 nmol of phosphate per min per g of yeast protein). It should also be noted that phenotype

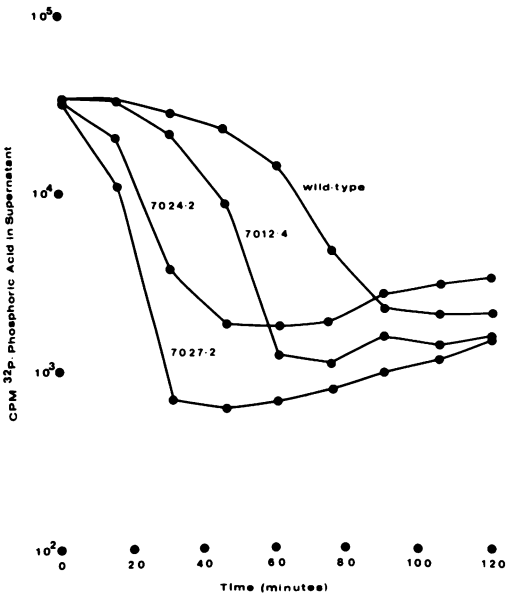

FIG. 1. *Phosphate uptake in S. cerevisiae isolates.*

7024-2, receiving 4.2 × 10^4 ergs per cuvette per 10 min, did not vary substantially in the average rate of phosphate uptake from 7012-4 (9.4 × 10^3 ergs per cuvette per 10 min).

Average rates of phosphate uptake for *S. cerevisiae* space flight phenotypes subjected to UV irradiation in space were approximately twice that of the wild type or ground control (Table 1). *Trichophyton terrestre* phenotype 7048-1 and *Rhodotorula rubra* phenotypes 1399-3, 1385-2, and 1529-2, exposed to space environmental parameters similar to those for *S. cerevisiae*, differed in phospholipid content when compared with the wild type (6; D. C. Deskins, M.S. thesis, Eastern Michigan University, Ypsilanti, 1978). Deskins noted that *R. rubra* phenotype 1399-3, which received 254 nm of UV irradiation, had the greatest variation in phospholipid content as compared with other phenotypes exposed to 280 and 300 nm of UV irradiation. The UV light wavelength rather than the energy level appeared to be the important factor in determining the degree of variation with respect to phospholipid content.

The cell membrane components are composed largely of phospholipids. A change in the amount and type of phospholipids present in a cell may significantly alter the ion transport system. The phosphate uptake mechanism involves the displacement of protons and potassium ions, along with an influx of $H_2PO_4^-$ (2, 4). From previous studies it is apparent that environmental parameters change the rate of phosphate uptake (1).

TABLE 1. *Phosphate uptake in S. cerevisiae and space-flown phenotypes*

S. cerevisiae isolate	Total phosphate utilized (nmol)	Period of phosphate uptake (min)	Yeast protein (g)	Avg rate of phosphate uptake (nmol of phosphate per min per g of yeast protein)	Wavelength of UV light (nm)
Wild type	43	91	3.3	0.14	
7027-2	43	31	3.6	0.38	254
7024-2	42	46	2.9	0.31	300
7012-4	42	61	2.6	0.27	280

Exposure to UV irradiation in space alters the phospholipid content of *T. terrestre* and *R. rubra* (6; D. C. Deskins, M.S. thesis, Eastern Michigan University, Ypsilanti, 1978).

The variation in phospholipid content in the selected yeast phenotypes was not traced to a specific mutation. However, UV irradiation at the energy levels and wavelengths examined in the Apollo 16 MEED does induce mutation (3, 7). Space parameters to which the yeast cells were exposed cannot be dismissed as factors leading to variation in phospholipid content of the phenotypic isolates.

S. cerevisiae exposure to UV irradiation in space causes rate changes involving phosphate uptake. Alteration of the phospholipid content may involve a change in cell membrane physiology affecting ion transport systems, including the phosphate uptake mechanism.

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