Absence of Photoreactivating Enzyme in
Candida albicans, Candida stellatoidea and Candida tropicalis

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In vitro assays demonstrate photoreactivating enzyme activity in extracts of Candida pseudotropicalis but not in extracts of C. albicans, C. stellatoidea or C. tropicalis.

Acknowledgement--These studies were aided in part by
a contract (AT-11-1)-1772 with the U. S. Atomic Energy
Commission.

The term photoreactivation refers to recovery by biological systems from detrimental effects of ultraviolet irradiation (uv) through exposure to visible or near visible light (4). Most commonly, the phenomenon reflects repair of uv-induced primidine dimers in deoxyribonucleic acid (DNA) through the activity of a specific light-activated enzyme (7). Sarachek and Ireland (6) examined those species of Candida most frequently associated with human disease for photorecovery from uv-inactivation and noted that the more pathogenic ones, C. albicans, C. stellatoidea and C. tropicalis do not photoreactivate whereas species of lesser pathogenicity, C. guilliermondii, C. parasilosis and C. pseudotropicalis (3) are photoreactivable. Failure of intact yeast cells to photoreactivate cannot be considered proof of their lack of photoreactivating enzyme. Miller, et al. (5) have shown that certain non-photoreactivable species of the perfect genus Hansenula do possess photoreactivating enzyme activity expressed in the ability of broken cell preparations to catalyze in vitro photorepair of uv-inactivated transforming DNA prepared from Haemophilis influenzae. To test for a possible cryptic presence of photoreactivating enzyme in C. albicans, C. stellatoidea or C. tropicalis, we have assayed extracts prepared from each of these species and from the photoreactivable species, C. pseudotropicalis, for light-dependent ability to restore transforming activity to uv-inactivated Haemophilis DNA.

The cultures examined in this study are listed in Table 1. The two strains of C. albicans used were provided by H. F. Hasenclever, National Institutes of Health, U. S. A.; the remaining test organisms were obtained from the American Type Culture Collections, Rockville, Maryland, U. S. A.

(ATCC strains) and the Northern Regional Research Laboratory, Peoria, Illinois, U. S. A. (NRRL strains). Haemophilis influenzae, Rd, was obtained from Jane K. Setlow, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U. S. A. A streptomycin-resistant substrain of Rd was obtained by transformation using a DNA preparation provided by J. K. Setlow.

Photorepair of DNA was followed in terms of restoration of the ability of uv-irradiated streptomycin-resistant DNA to transform streptomycin-sensitive cells of H. influenzae to streptomycin-resistance.

Methods for preparation of cell-free homogenates and transforming DNA as well as the procedures for uv-irradiation of DNA and for transformation experiments have been described previously (5). Protein concentrations of crude cell extracts ranged from 28 to 80 mg/ml. The extracts were routinely adjusted with 0.067 M phosphate buffer containing 1 mM EDTA to yield 10 mg/ml protein for use in transformation assays. When extracts used at this concentration failed to show photoreactivating activity, the negative result was confirmed by testing at the maximum protein concentration available. For photoreactivation, mixtures of cell extract plus uv-treated DNA were illuminated continuously in a 25°C water bath by means of a Sylvania 400 watt sun lamp, model SFL-150-101 at a distance of 35 cm. All samples were equilibrated for 30 minutes at 25°C prior to illumination. In every cell, no significant difference in transforming activity of DNA was detectable between 20 minutes and 60 minutes of illumination. Controls for each experiment included (i) an extract plus irradiated DNA mixture held in the dark, (ii) an extract plus unirradiated DNA mixture subjected to illumination and (iii) an illuminated solution of DNA in 0.067 M phosphate buffer containing 1 mM EDTA.

Table 1 records typical results of assays for photoreactivating enzyme activities. It is seen that extracts of C. pseudotropicalis promote as much as 35 percent photorecovery of the transforming capability of DNA. In contrast, no activity is detectable in extracts of the other species. Thus, the inability of intact cells of C. albicans, C. stellatoidea or C. tropicalis to photoreactivate is attributable to their lack of photoreactivating enzyme. This mutual enzymatic deficiency is consonant with the substantial body of other biochemical and serological evidence of a very close phyletic relationship between these three species (1,2,8).

Table 1. Photoreactivation of uv-irradiated-transforming DNA by cell-free extracts*

<u>Candida</u> species	Strain	Experiment	Number of transformants/ml in:			
			Unirradiated DNA + crude extract (illuminated)	Irradiated DNA@ + buffer (illuminated)	Irradiated DNA + crude extract (dark)	Irradiated DNA + crude extract (illuminated)
<u>Candida albicans</u>	207	#1	1.19×10^5	4.6×10^3	5.0×10^3	3.6×10^3
		#2	9.6×10^3	1.1×10^2	4.7×10^2	4.2×10^2
	526	#1	1.42×10^5	3.2×10^3	9.4×10^3	6.8×10^3
		#2	1.23×10^4	3.3×10^3	3.4×10^2	4.9×10^2
<u>pseudotropicalis</u>	ATCC 8553	#1	1.25×10^4	1.0×10^2	2.0×10^2	3.4×10^3
		#2	1.20×10^4	1.7×10^2	6.0×10^2	4.8×10^3
<u>stellatoidea</u>	ATTC 11006	#1	1.59×10^5	4.3×10^3	6.0×10^3	3.8×10^3
		#2	1.05×10^4	1.7×10^2	2.0×10^2	2.8×10^2
	NRRL-Y 2361	#1	1.11×10^5	1.9×10^3	2.6×10^3	2.3×10^3
		#2	9.73×10^3	1.0×10^2	8.5×10^2	8.2×10^2
<u>tropicalis</u>	ATTC 1369	#1	8.10×10^4	2.0×10^3	3.6×10^3	5.9×10^3
		#2	2.33×10^5	5.2×10^3	7.3×10^3	9.7×10^3
	NRRL-Y 1410	#1	5.00×10^4	2.0×10^3	3.0×10^3	6.3×10^3
		#2	1.60×10^4	5.0×10^2	6.9×10^2	7.3×10^2

* The total viable cell count of streptomycin-sensitive Haemophilis influenzae varied from 1.5×10^8 to 7.4×10^8 cells/ml.

@ uv irradiation of H. influenzae DNA resulted in 95.2-99.8 percent inactivation of transforming activity.

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