Final Technical Report to the U.S. Atomic Energy Commission
contract AT(11-1)-1772

Genetics of *Candida albicans*: Nutritional mutagenesis and
comparative susceptibility to ultraviolet irradiation

at

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Our AEC contract AT(11-1)-1772 has been directed toward (i) identifying the mechanisms available to the ubiquitous, pathogenic yeast, Candida albicans, for coping with ultraviolet irradiation (uv) and (ii) evaluating the possible systematic distribution of various postirradiation recovery mechanisms among the taxonomically well defined classes of sporogenous and asporogenous yeasts. The salient findings can be summarized as follows:

I Radiobiology of C. albicans

Candida albicans does not photoreactivate. Because of the small quantity of DNA per cell and the difficulties in labeling the pyrimidines of yeast DNA (i.e. absence of thymidine kinase), it is not feasible to relate modifications in the dark recovery of any yeast to DNA excision or recombination systems by the direct quantitative physicochemical analyses applicable to bacteria and some eucaryotes. Inferences regarding the mechanisms of dark recovery in such organisms are traditionally based on parallels in their responses to nutritional and physical postirradiation conditions having known effects on repair processes in bacteria. By these criterion we have obtained evidence of postreplicative DNA repair but not excision repair in C. albicans. On the other hand, we have established that dark recovery of C. albicans is enhanced by post-uv growth (i) at 25°C rather than 37°C, (ii) on minimal rather than enriched medium or (iii) in the presence of sterols or long chain fatty acids. These effects are noted for cellular inactivation but not mutagenesis and are restricted to cells which are non-dividing at the time of irradiation. The composite of our observations indicate that these three forms of dark recovery reflect the occurrence of reparable, uv-induced perturbations of the cell membrane which are of critical

importance in the initiation of the budding process.

II Systematics of Post-UV Recovery Processes In Yeasts

A survey of eighty-three species representing 12 perfect and 10 imperfect genera established that the ability to photoreactivate lethal uv damage is a genus dependent trait in yeasts. Photoprotection or indirect photoreactivation were not detected in these studies. An exhaustive survey of the genus Hansenula, the best defined of the yeast genera, systematically, further revealed that the magnitudes of the photoreactivable sectors of species correspond well with their phylogenetic placement within the genus. Parallel findings were subsequently made with the cognate, but more diffuse, genus Pichia. Thus selection for photoreactivability appears to be a significant feature of the evolutionary divergence of yeasts. In vitro assays for photoreactivating enzyme in Hansenulas revealed no correlation between the photoreactivable sectors of cells and their enzyme contents. Thus, the systematic distribution of photoreactivable sectors among the Hansenulas most probably represents either (i) features of nuclear organization that affect accessibility of photoreactivating enzyme to uv-damaged DNA or (ii) differences in the amounts of genetic and non-genetic damages contributing to cellular inactivation by UV.

Photoreactivation action spectra were determined on representative species of the genera Hansenula, Kluyveromyces, Nadsonia, Saccharomyces and Trigonopsis. The spectral requirements for photoreactivation are genus dependent. Species within the same genus have like spectra and the spectra of related genera are more similar than those of unrelated ones. Thus, the genetic information determining the spectral properties of photoreactivating enzyme does not diverge during speciation but does in

the process of differentiation of genera. These observations have important implications for the use of photoreactivation action spectra in clarifying ambiguous taxonomic situations among the yeasts and as a probe for identifying systematic relations among the higher taxa of these fungi.

Of the seven species of Candida implicated in human disease, the three most pathogenic ones, C. albicans, C. stellatoidea and C. tropicalis, lack photoreactivating enzyme whereas the remaining four species do photoreactivate. This is consistent with other lines of serological and physiological evidence of strong systematic affinity between the three highly pathogenic species. It also conforms with the general observation that highly pathogenic bacteria are not photoreactivable (e.g. Clostridia, Corynebacterium, Haemophilis, Neisseria, Streptococci). Each species of Candida exhibits a specific profile with respect to photoreactivability and temperature, nutritional and lipid dependent dark recoveries from uv irradiation. These profiles have proven useful as diagnostic tools in identifying Candida species among clinical isolates and in confirming the putative perfect forms of Candida species among yeasts consigned to other sporogenous genera.

Reprints or manuscripts of papers concerning the above results which are published or in press are appended to this report. Manuscripts of papers in preparation will be forwarded as soon as completed.

M.S. THESES completed under contract AT(11-1)-1772

- D.L. Busbee. Inactivation of Candida albicans by ultraviolet radiation. 1968.
- R.V. Goering. Evidence for temperature-sensitive non-genetic repair in Candida albicans. 1969.
- N.P. Higgins. Effects of ergosterol, palmitic acid and related lipids on survival of ultraviolet irradiated cells of Candida albicans. 1970.
- C.J. Faruham. In vitro photoreactivation in species of Hansenula. 1971.
- J. Brammer. Effects of nutrition and growth temperatures on recovery from ultraviolet radiation by pathogenic species of Candida. 1973.
- R.W. Pettriess. Influences of cellular susceptibility to amphotericin B and of postirradiation growth conditions on inactivation of Candida albicans by ultraviolet radiation. 1974.

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- Miller, G.R., and Sarachek, A., Absence of photoreactivating enzyme in *Candida albicans*, *Candida stellatoidea* and *Candida tropicalis*. *Infection and Immunity*, accepted for publication.
- Sarachek, A. and Pettriess, R.W., Influences of cellular susceptibility to amphotericin B and of post-irradiation growth conditions on inactivation of *Candida albicans* by ultraviolet radiation. *Mycopath. et Mycol. Appl.*, accepted for publication.
- Sarachek, A. and Bish, J.T., Phylogenetic implications of the action spectra for photoreactivation of selected ascomycetous yeasts. *In preparation.*
- Sarachek, A. and Brammer, J., Applicability of photoreactivation and dark recovery processes following ultraviolet irradiation to identification of species of *Candida* and their perfect forms. *In preparation.*