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Use of Candida albicans for testing mutagenic and phototoxic substances

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Abstract: *Usefulness of candida albicans for rapid screening of mutagenic agents and sun screeners have been explored by using UV induction. The study findings clearly show that Candida albicans will serve as reliable tool for the above study which we have validated and confirmed using the battery of sunscreeners such as avobenzone, octylmethoxycinnamate, benzophenone. We have further counter checked the study model using the extracts of Psoraleacorylifolia. Findings are presented in the paper.*

Keywords,*Sun screeners, Candida, Germ tube, Avobenzone*

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I. Introduction

*Candida albicans*is yeast like fungi usually present in human body as commensal flora [1, 2]. However this organism is notorious for its opportunistic pathogenic ability especially in immune compromised patients [3,4]. The life threatening diseases due to *Candida albicans*is quite common these days due to large pool of patients suffering from various auto-immune disorders [5].

*Candida albicans*has been used for several researches to understand the drug resistance pattern and genetic studies. Recently Aruna et al., has established the usefulness of *Candida albicans*for screening anti-diabetic ingredients by exploiting the sugar assimilation pattern [6].

The present study deals with the response of *Candida albicans*to UV irradiation and how useful will be the morphological/cellular changes in Candida albicans in response to UV for predicting the effect of ingredients/products on DNA. Findings are presented in the paper.

II. Materials and Methods

Twenty four hour grown *Candida albicans*culture was inoculated on toSabouraud's dextrose agar and allowed the culture to settle on the plate for 15 minutes. After which the plate was exposed to 3 joules/cm2 of UV for 5, 10 and 15 minutes. After UV exposure the plates were incubated at 37C for 24 hours. The total number of colonies grown and the number of mutant colonies were counted and compared with control plate. The mutant colonies were sub-cultured to check mutant reversion. The culture was examined microscopically to characterize the morphological changes in the cell. The above experiments were done in quadruplet and the concordance in the findings was established.

III. Result

84% reduction in CFU of *Candida albicans*was observed in 5 minutes exposure to UV. When the exposure time was increased to 10 minutes near 100% reduction was observed. However none of the survived colonies showed any mutant property. Table- 1

Out of 1600 colonies withstood 5 minutes UV exposure, 5 colonies showed mutation. When the mutant colonies were sub –cultured 3/5 colonies showed mutant reversal. Table- 1

Description	Number and percentage reduction in CFU
No of CFU's of *Candida albicans*in control	10,000
No of CFU's of *Candida albicans*in UV treatment 5 minutes 10 minutes 15 minutes	 1600 (84%) 2 (99.9%) Nil
No of Mutant cells in 5 minutes treatment	5
Mutant reversal	3 (60%)

Table-2-Microscopic and growth characteristics of *Candida albicans* in response to UV treatment

UV untreated *Candida albicans* cells showed typical characteristics and germ tube formation when grown in serum. The *Candida albicans* cells that withstood 5 minutes UV exposure were microsized and showed great aggregation/clumping indicating the likely possibility of cell wall lysis at the point of contact between cells. The mutated colonies did not produce germ tube. However on subculture they showed mutant reversion at colony morphology which was further supported by the formation of germ tube in sparse manner. Table- 2

Description	**Microscopic characteristics**	**Germ tube formation**
Untreated *Candida albicans* cells	Abundant, individualized uniform sized cells with even distribution	Abundant
5 min UV exposure (Non- Mutated colony)	Micro sized cells showing great aggregation/clumping shown by small group of colony	Sparse
5 min UV exposure (Mutated colony)	Abundant micro sized cells showing great aggregation/clumping	No germ tube formation
Colony that showed Mutant reversion	Abundant micro sized cells showing great aggregation/clumping	Sparse

Response of *Candida albicans* cells to UV under various conditions.

Candida albicans cells grown in avobenzone, Octylmethoxycinnamate, Benzophenoneand then expose to UV did not show any characteristic change, reduction in CFU and any aberration in germ tube formation. On the contrary a Candida albicans cell grown in *Psoraleacorylifolia* and then exposed to UV has resulted in 100 % inhibition in growth. Table- 3

Description	Microscopic characteristics	Germ tube formation
Untreated Candida cells	Abundant, individualized uniform sized cells with even distribution	Abundant
Candida cells grown in media with Avobenzone- 5 min UV exposure	Abundant, individualized uniform sized cells with even distribution	Abundant
Candida cells grown in media with Octylmethoxycinnamate - 5 min UV exposure	Abundant, individualized uniform sized cells with even distribution	Abundant
Candida cells grown in media with Benzophenone - 5 min UV exposure	Abundant, individualized uniform sized cells with even distribution	Abundant
Candida cells grown in media with Psoraleacorylifolia - 5 min UV exposure	No growth	No growth

IV. Discussion

UV induced mutation in *Candida albicans* is well known. However the detailed account on the morphological and microscopic characteristics of the cells after UV exposure the time required to produce such change and the possibility of mutation and the possibility of mutation reversal are not clearly understood. Such understanding would help us to use the UV response pattern of *Candida albicans* as an excellent tool to screen various mutagens and their likely effect on the microscopic characteristics of *Candida albicans*. Further such tool also would help us to develop potent anti-mutagens. Today no reliable cost effective and less time consuming method is available and hence the above study assumes greater importance.

The present study has shown that 5 minutes UV exposure was sufficient to cause growth inhibition to an extent of 84%. Interestingly most of the cells that survived UV exposure did not show any signs of mutation. The cells that showed signs of mutation were not permanent and all microscopic characteristics got reversed in subsequent sub culture.

The mutant reversed cells were tested with avobenzone, benzophenone, octylmethoxycinnamate, and then exposed to UV. Interestingly none of the cells showed any mutation strongly indicating the UV protecting property of above sun screeners. When we exposed the cells to UV with 1% *Psoraleacorylifolia* extract, all the cells showed mutant response validating the UV sensitizing property of*Psoraleacorylifolia* extract. This experiment we have done to validate the robustness and usefulness of the above method that we have developed through our intense research. Although this method may require some more scientific validation and confirmation but the above method will be undoubtedly useful for rapid screening of sun screeners, mutants and other photo toxic agents.

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