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Cobalt 60 Radiation and Growth of *Candida* Species

Kobalt-60-Bestrahlung und das Wachstum von *Candida*-Arten

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Key words: *Candida* – cobalt 60 – pseudomycelium – blastospores
Schlüsselwörter: *Candida* – Kobalt-60 – Pseudomyzel – Blastosporen

Summary: Eight pathogenic *Candida* species were exposed to ^{60}Co radiation and examined for variation in growth and development. All isolates demonstrated accelerated pseudohyphal growth and blastospore production at 10,000 rads compared with controls receiving no gamma radiation. Growth gradually decreased with increased ^{60}Co exposure, and minimal recovery occurred with four species at 2.4 million rads.

Zusammenfassung: Acht pathogene *Candida*-Arten wurden ^{60}Co-Strahlung ausgesetzt und auf Normabweichungen in Wachstum und Entwicklung untersucht. Alle Isolate zeigten beschleunigtes Pseudomyzel-Wachstum und beschleunigte Blastosporen-Bildung bei 10 000 rad im Vergleich zu den Kontrollen, die keine Gammastrahlung erhielten. Das Wachstum nahm mit erhöhter ^{60}Co-Exposition ab, und eine geringgradige Erholung wurde bei vier Arten bei einer Bestrahlungsdosis von 2.4 Millionen rad beobachtet.

Introduction

Previous studies have shown that radiation affects the growth dynamics of various microorganisms (1, 7). In some cases the organism had an increased growth rate as radiation levels increased (2, 8). The current study is concerned with the growth dynamics and morphology of various species of *Candida* when exposed to ^{60}Co gamma radiation. *Candida* cells were exposed to ^{60}Co radiation, including levels received by oncology patients during treatment, and levels above and below therapeutic doses.

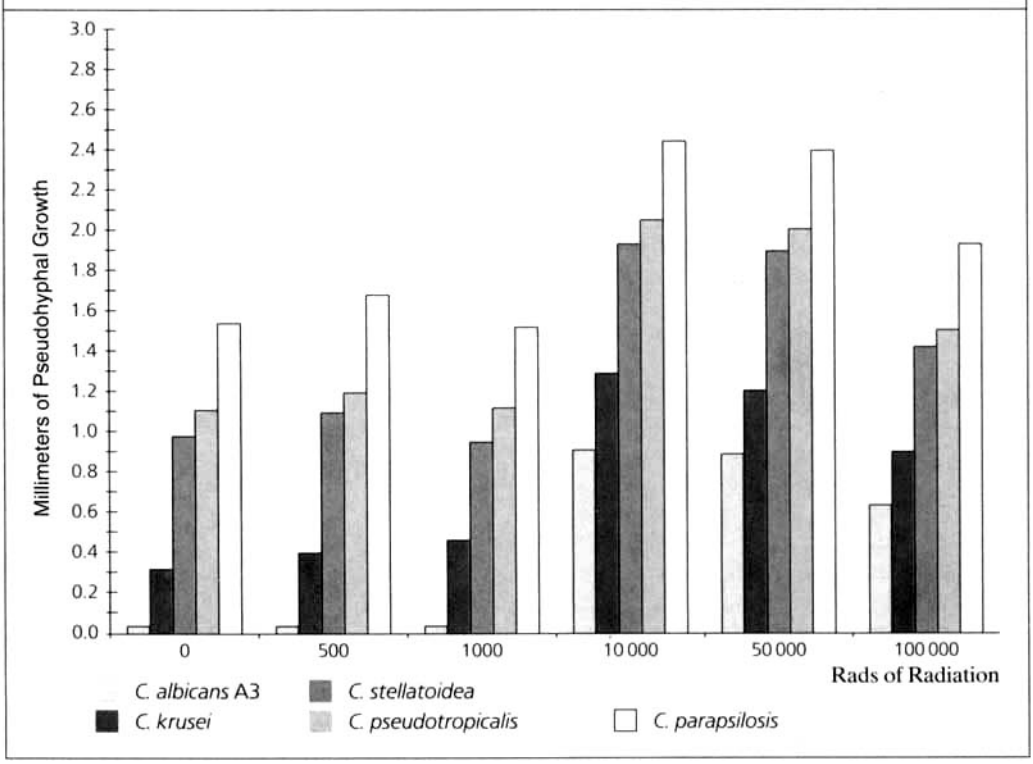
Materials and Methods

The ATCC *Candida* isolates selected for this study included *C. albicans* 10231, *C. guilliermondii* 6260, *C. krusei* 6258, *C. lusitaniae* 34449, *C. parapsilosis* 10232, *C. pseudotropicalis* 4135, *C. stellatoidea* 11006, *C. tropicalis* 750, and two patient isolates of *C. albicans*. Actively growing stock cultures were maintained on Sabouraud dextrose agar (Difco). Two hours prior to ^{60}Co exposure for zero days pseudohyphal growth at the time of irradiation, isolates were transferred to Cornmeal agar (Difco) plates using the Dalmau technique (3). A 22 x 50 mm no. 1 Corning cover glass was placed over a single streak of the isolate on the agar surface. Replicates of

Table 1: ^{60}Co exposure levels for *Candida* species

Dose rate	x	Irradiation time	=	Dosage received	Distance from source
250 rads/min		2 min		500 rad	82 cm
500 rads/min		2 min		1,000 rad	30 cm
5,000 rads/min		2 min		10,000 rad	9.5 cm
50,000 rads/hr		1 hr		50,000 rad	40 cm
100,000 rads/hr		1 hr		100,000 rad	25 cm

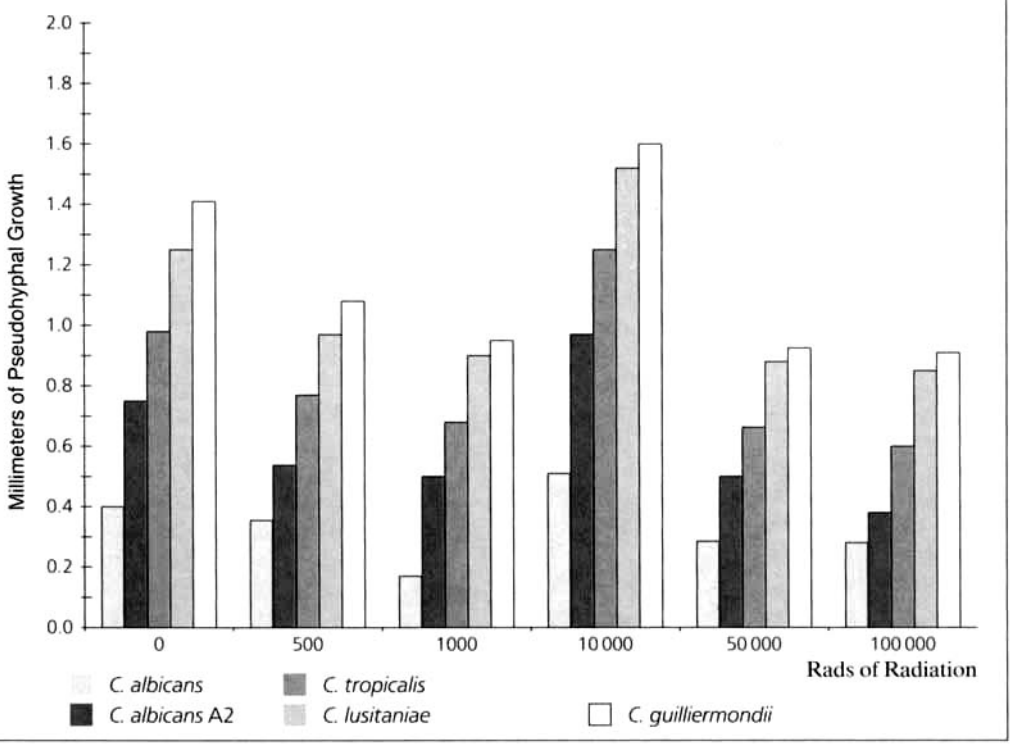
Graph 1: Pseudohyphal growth greater than control at 10,000 rad ^{60}Co and at other exposure levels in selected *Candida* isolates.



four plates were made of each organism at each radiation level examined. The Dalmau plates were placed within the Michigan Memorial Phoenix Project Cobalt 60 Source Irradiator. *Candida* isolates received gamma radiation at cumulative dose levels of 500 to 100,000 rads (Table 1). In addition, the *Candida* species were exposed to radiation levels up to 2.4 million rads for tolerance studies. No change was noted in the growth medium due to irradiation.

Control Dalmau plate isolates received no ^{60}Co exposure. All plates were incubated at 24 °C before and after irradiation. The Dalmau plate technique assists *Candida* species identification according to specific growth of pseudohyphae, blasto-

Graph 2: Pseudohyphal growth greater than control only at 10,000 rad ^{60}Co in selected *Candida* isolates.



spore production, and the presence of chlamydospores (6). The Dalmau method and Cornmeal agar stimulate pseudohyphal development in species of *Candida*. Data collected refer to pseudohyphal length and blastospore production of each *Candida* isolate according to ^{60}Co radiation exposure.

Measurements were made on the pseudohyphal length of each isolate two days after ^{60}Co exposure. Averages were taken on 40 pseudohyphal strands for each test system of each species at the predetermined doses. Blastospore production according to each test system was also recorded. Yeast growth was examined using a Leitz Wetzlar Ortholux microscope at 35 and 100 magnification. Measurements were converted into mm length for tabulations.

Results

In Graph 1, pseudohyphal growth for 5 *Candida* species is compared to rads of radiation received. The isolates include *C. albicans* (patient isolate A3), *C. krusei, C. stellatoidea, C. pseudotropicalis,* and *C. parapsilosis.* No pseudohyphal formation occurred in isolate A3 at 0, 500 or 1,000 rads. Greatest production of pseudohyphal growth for all 5 isolates occurred at 10,000 rads. A growth decrease occurred from 10,000 rads to 50,000 and 100,000 rads respectively, however, these highest dose radiation levels produced growth greater than that at zero radiation levels. At 500 and 1,000 rads, pseudohyphal growth either equalled or was generally slightly greater than control for each of the *Candida* isolates.

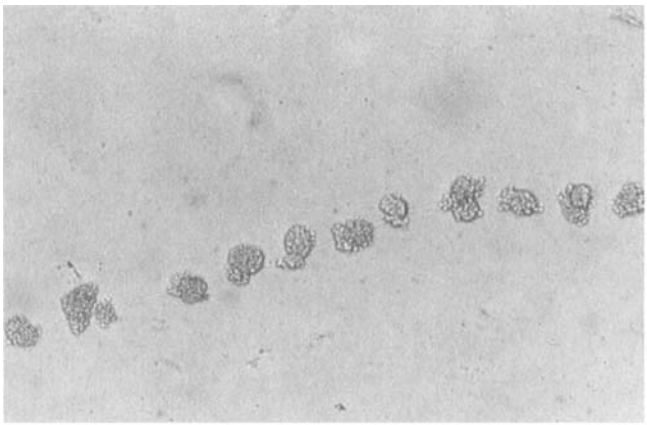


Fig. 1: *C. albicans* control without radiation exposure showing pseudohyphae and blastospore production at 2 days growth. 470 x.

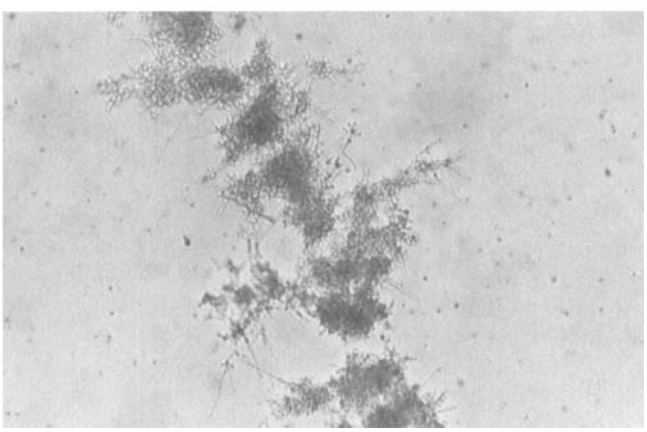


Fig. 2: *C. albicans* after 10,000 rads radiation exposure showing pseudohyphae, blastospores, and germinating blastospores at 2 days growth. 470 x.

Graph 2 presents the pseudohyphal growth of *C. albicans, C. albicans* (patient isolate A2), *C. tropicalis, C. lusitaniae,* and *C. guilliermondii* according to exposure levels of radiation in rads. Similarly as in Graph 1, isolates presented in Graph 2 also produced the greatest pseudohyphal growth at 10,000 rads exposure. However, the organisms presented in Graph 2 only exceeded the control group in growth at 10,000 rads. All additional exposure levels, including 500, 1,000, 50,000, 100,000 rads and higher exhibited growth rates somewhat less than that of the control group receiving no gamma radiation.

Additional exposures at increments of 200,000 rads each above 100,000 rads were made for maximum tolerance studies for *Candida* above dose levels issued for patients. No growth occurred for *C. albicans, C. albicans* A2, and *C. guilliermondii* at 1.4 million rads, and for *C. pseudotropicalis* at 1.6 million rads. At 2.4 million rads, *C. albicans* A3 and *C. parapsilosis* were inhibited, however, very slight recoveries in pseudohyphal growth occurred with *C. krusei, C. lusitaniae, C. stellatoidea,* and *C. tropicalis.*

Blastospore formation occurs at septal divisions along pseudohyphal strands, and blastospore production varies according to specific species of *Candida* (5, 6). In the current studies, the abundance of blastospores varied according to growth rates of pseudohyphae at each exposure level of gamma radiation. At two days growth, a

pseudohyphal strand of *C. albicans* is shown in Figure 1 with normal blastospore production, and no blastospore germination. After exposure to 10,000 rads ^{60}Co radiation, *C. albicans* at two days significantly increased blastospore production, lateral pseudohyphal growth, and blastospore germination 2 to 4 times greater than normal growth rates (Figure 2).

Discussion

Candida species can be isolated as normal flora on the human body, located in the upper respiratory tract, intestinal tract, and vaginal tract of healthy individuals (4). Yeast species selected for the current study can cause disease in humans, given the opportunity and the proper environmental conditions. Candidosis is a major problem in oncology patients and ^{60}Co radiation is a frequent treatment method for these patients. With Hodgkin's disease, lymphomas, and other wide-spread malignancies, large areas of the body receive gamma radiation in treatment regimes. Major hospitals routinely monitor oncology patients for yeast cell levels, and precautions are taken to reduce a sudden occurrence of thrush or candidosis.

Candida, present as normal flora in the cancer patient, may produce more pseudohyphae and blastospores after the patient undergoes ^{60}Co radiation treatment. Current studies indicate that the irradiated yeasts in vitro exhibited increased growth after being exposed to radiation levels between 500 and 10,000 rads. The human radiation doses received in regular treatment regimes are 4,500 to 6,000 rads. The experimental environment under which the yeasts were grown had reduced oxygen using the Dalmau plate method. *Candida* in vivo is also in a reduced oxygen environment. Cancer patients, with a depressed immune system, suffer from a variety of secondary infections, including candidosis. The current study identifies increased *Candida* growth under ^{60}Co radiation exposure. The increase in candidosis may not only be caused from the depressed immune system, but candidosis incidence may also be directly stimulated by the ^{60}Co gamma radiation treatment. As the radiation stimulates pseudohyphal production, the fungus also produced increased numbers of blastospores.

Growth of *Candida* species was stimulated to above normal growth rates from gamma radiation doses in the same range as those received by oncology patients. Routine clinical procedures should include pretreatment of patients with antifungal drugs before and during radiation therapy if the current in vitro findings reflect in vivo *Candida* involvement.

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