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Photoprotective role of carotenoids in yeasts: Response to UV-B of pigmented and naturally-occurring albino strains

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ABSTRACT

In this work, the photoprotective role of carotenoids in yeasts was analysed by contrasting the responses to UV-B of pigmented and naturally occurring albino strains of *Sporobolomyces ruberrimus* and *Cystofilobasidium capitatum* in different conditions. Albino and pigmented strains were confirmed to be conspecific by PCR fingerprinting and rDNA sequencing. Experimental exposure to UV-B conducted with both yeast species showed that the pigmented strains were more tolerant to UV-B than the albino strains and that the increment in carotenoid contents during the stationary growth phase enhance survivorship. These results indicated that carotenoid pigments afford UV-B protection in yeasts.

1. Introduction

Natural levels of ultraviolet radiation reaching the earth's surface may induce severe damage in most organisms [1]. The impact of UV-R on organisms may have an indirect effect by promoting the formation of reactive oxygen species (ROS), which are highly toxic, or directly affecting intracellular structures by photolysis. This direct effect is caused mainly by UV-B (280–315 nm), which damages DNA by generating two types of mutagenic lesions, the cyclobutane pyrimidine dimers and the pyrimidine–pyrimidone (6–4) dimers [2].

Carotenoids are liposoluble tetraterpene-type molecules which are generally red to yellow, due to the presence of several conjugated double bonds that act as chromophores [3]. In Fungi, their fundamental role is to provide protection against reactive oxygen species (ROS), specifically $^1O_2$. Furthermore they provide indirect protection against both UV-A (315–400 nm) and visible light (photosynthetically active radiation, PAR 400–700 nm) by means of ROS quenching [4]. Several yeast species are known to accumulate carotenoid pigments as secondary metabolites. In these microorganisms, carotenoid synthesis is associated with growth. Maximum carotenoid accumulation is observed in stationary phase in relation with cell ageing, and is probably a general mechanism of defence against oxidative stress [5–7]. The synthesis of torularhodin, torulene, and β-carotene is common in several genera of *Rhodotorula, Sporobolomyces,* and *Cystofilobasidium* [8]. In these microorganisms, carotenoids may contribute to preserve the viability of ageing cells by quenching oxygen radicals, possibly compensating for their lack of antioxidant enzymes [9].

The synthesis of photoprotective compounds is a common response of several organisms when exposed to high irradiation; however, there are few reports of photoprotection afforded by carotenoids in microorganisms in general [10,11] and yeasts in particular [12]. Moreover, there is still controversy regarding the photoprotection efficiency of carotenoids in the range of UV-B wavelengths [3,13].

This work focuses on the photoprotective role of carotenoids in yeasts by comparing the survival to UV-B of pigmented and naturally-occurring albino strains of two species in different conditions. Three experiments were carried out. The first experiment analysed the survivorship of albino and pigmented strains exposed to UV-B for different lengths of time. The second trial tested the effect of UV-B on the survivorship of strains at different growth phases, and related survival to different carotenoid concentrations at each phase. Finally, the third experiment evaluated the production of carotenoids by photo-induction, and the effect of UV-B on yeast survival.

2. Materials and methods

2.1. Yeast characterization

Two basidiomycetous yeast species were studied: *Sporobolomyces ruberrimus* and *Cystofilobasidium capitatum*. For each of these

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Table 1
Species, strain number, origin, presence of carotenoids and nucleotide sequence genbank accession numbers of the six yeast strains used in this study.

Species and strain N°	Origin	CAR	Genbank	
			D1D2	ITS
Sporobolomyces ruberrimus				
CRUB 1040 (Wild)	Ortiz Basualdo lake, Patagonia, Argentina	+	AY619557[a]	–
CBS 7500[b] (Type)	Air, Japan	+	AF070442[a]	AY070004[c]
CBS 7501[a] (Albino)	Culture of CBS 7500[T]	–	AF189993[a]	AY015436[c]
Cystofilobasidium capitatum				
CRUB 1047 (Wild)	Nahuel Huapi lake, Patagonia, Argentina	+	AY849807[b]	–
CBS 6358[b] (Type)	Zooplankton, Antarctica.	+	AF075465[b]	AF139627[d]
CBS 7420[a] (Albino)	Bird *Larus marinus*	–	AF075466[b]	AF444300[d]

CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Utrecht, The Netherlands.
CRUB, Centro Regional Universitario Bariloche, Bariloche, Argentina.
CAR, Carotenoids.
GenBank, accession numbers.
100% nucleotide similarity between the sequences sharing the same letter.
[a] Nonpigmented.
[b] Type strain.

two species, three strains were used (details are given in Table 1). The synthetic oligonucleotide $(GTG)_5$ and the core sequence of the phage M13 (GAGGGTGGCGGTTCT) were employed as primers in PCR assays. Procedures were applied as reported in Libkind et al. [14]. Further characterization was obtained by sequence analysis of the 26S rDNA partial sequence, and the ITS region, according to Sampaio et al. [15].

2.2. UV-B effect on survival curves of albino and pigmented strains

Each of the six strains were irradiated at stationary phase of growth (66 h of culture for *S. ruberrimus* and 72 h for *C. capitatum*). Yeasts were propagated in Erlenmeyer flasks with 100 mL of MMS, in an incubator–shaker at 180 rpm and 22 °C under regular laboratory illumination. Cells were harvested, rinsed twice with sterile distilled water, and transferred to quartz test tubes containing 20 mL of sterile distilled water. A final cell concentration of 2×10^5 cell mL^{-1} was reached. Quartz tubes were exposed to a Spectroline XX15-B UV-B lamp covered with cellulose acetate filter, in a dark chamber, producing almost no radiation below 295 nm. Although there was an output of short UVA wavelengths (315–340 nm), this contribution was very small respect to UVB and in comparison with natural levels [16]. Radiation spectrum and intensity were measured with a USB2000 Fiber optic spectroradiometer (Ocean Optics Inc). Before each experiment, the filter was changed and preburned for 120 min [17,18] to ensure the radiation pattern observed in Fig. 1A. Experiments were run in triplicate. Samples were taken every 10 min. During exposure tubes were constantly stirred.

2.3. UV-B effect on albino and pigmented strains at different growth stages

In the second experiment yeast cells at exponential (18 h of culture for *S. ruberrimus* and 24 h for *C. capitatum*) and at stationary (66 h for *S. ruberrimus* and 72 h for *C. capitatum*) growth phases were irradiated. Culture and irradiation conditions were set up as mentioned above. *S. ruberrimus* cells were irradiated to UV-B for 10 min, while a 20 min exposure was applied to cells of *C. capitatum*. Total carotenoid concentration was measured for both growth phases.

2.4. UV-B effect on albino and pigmented strains photostimulated with PAR

In the third experiment, we attempted to enhance carotenoid production of yeast strains by culturing in solid MMS, under PAR, in an environmental test chamber (SANYO MLR 350) for 3 day, applying a photoperiod of 12:12 light:dark [19]. Light was provided by 10 white light fluorescent tubes (SANYO, 40 W), resulting in the pattern of radiation shown in Fig. 1B. Dark controls, wrapped in aluminium foil were also included. After photo-induction, colonies were suspended in distilled water and transferred to quartz test tubes, as described for the first and second experiments. Total carotenoid concentration was measured for both treatments.

2.5. Culture media

Minimal medium salts (MMS), either liquid (broth) or solidified by the addition of 15 g L^{-1} of agar. MMS was prepared with

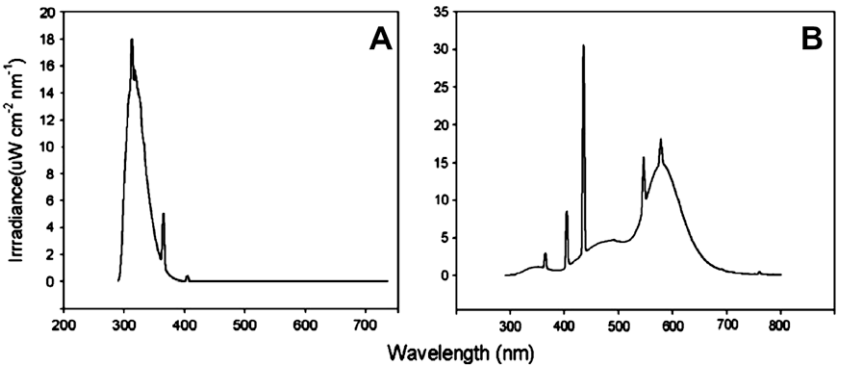

Fig. 1. Radiation spectra of the different light treatments applied: A, Spectroline XX15-B UV-B covered with a cellulose acetate (UV-C filter); B, PAR provided by 10 white light fluorescent tubes (SANYO, 40 W).