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Constitutive and UV-inducible synthesis of photoprotective compounds (carotenoids and mycosporines) by freshwater yeasts

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Twelve yeasts isolated from lakes of Northwestern Patagonia, Argentina, belonging to eight genera (*Sporobolomyces*, *Sporidiobolus*, *Rhodotorula*, *Rhodosporidium*, *Cystofilobasidium*, *Cryptococcus*, *Torulaspora*, and *Candida*) were analysed for their ability to produce photoprotective compounds. For this purpose, three laboratory experiments were performed to study the effect of photosynthetically active radiation (PAR) and PAR in combination with UV radiation (PAR + UVR) on the production of carotenoids and mycosporines. The synthesis of carotenoid compounds was clearly stimulated in six out of nine red yeast strains tested upon exposure to PAR or PAR + UVR; however, the latter conditions produced a stronger response than PAR alone. The increase in carotenoids in the red strains under PAR + UVR irradiation showed a negative exponential relationship with their basal carotenoid content, suggesting that cells with higher constitutive levels of carotenoids are less responsive to induction by PAR + UVR. Three red yeasts, *Rhodotorula minuta*, *Rh. pinicola*, and *Rhodotorula* sp., and the colourless *Cryptococcus laurentii* produced a UV-absorbing compound when exposed to PAR or PAR + UVR. This compound showed an absorption maximum at 309–310 nm and was identified as mycosporine-glutaminol-glucoside (myc–glu–glu). In these strains, exposure to PAR or PAR + UVR resulted in elevated concentrations of both carotenoids and myc–glu–glu. This is the first report on the production of mycosporines by yeasts. All strains that developed under PAR + UVR were able to synthesise carotenoids either constitutively or in response to PAR exposure, and a few of them also produced myc–glu–glu when exposed to PAR. Collectively, our results suggest that the presence of carotenoids, either alone or in combination with mycosporines, are required for sustaining growth under exposure to PAR + UVR in the freshwater yeast strains studied.

Introduction

The occurrence of photo-protective compounds (PPCs) is widespread among aquatic organisms. Their presence has been shown to prevent photodamage in many aquatic organisms that are regularly exposed to high levels of ultraviolet radiation and visible light (hereinafter UVR and PAR, respectively). The mechanisms by which PPCs protect the organisms from damaging UVR may be either direct (*i.e.* optical screening of UV wavelengths) or indirect [*i.e.* quenchers and scavengers of highly toxic reactive oxygen species (ROS)]. In addition, carotenoids are known to be photoinducible in several algae species and also have been suggested to play a role in photoreactivation.[1]

The major groups of PPCs among aquatic organisms are melanins, carotenoids, and mycosporine-like amino acids (MAAs). The ability to synthesise PPCs varies among phylogenetic lineages. For example, animals produce melanins, but lack the metabolic pathways to *de novo* synthesise carotenoids and MAAs. In fact, carotenoid synthesis is exclusive to plants and microbes, while the synthesis of MAAs is apparently restricted to organisms possessing the shikimic acid pathway, which is lacking in all metazoa. Despite such metabolic deficiencies, carotenoids and MAAs are present in many invertebrates and vertebrates because they obtain these compounds from the diet or through symbiotic associations.[2]

Among heterotrophic organisms, several yeast genera (*e.g.* *Rhodotorula*, *Sporobolomyces*, *Rhodosporidium*, *Cryptococcus*, *Phaffia*) produce carotenoid pigments such as β-carotene, torulene, torularhodin, and astaxanthin.[3–5] The presence of mycosporines in yeast has not been reported so far, even though they have been found in several filamentous fungi.[6,7]

In this study, we analyse the production of carotenoids and UV-absorbing compounds in twelve yeast strains isolated from freshwater systems located within the Nahuel Huapi National Park (Patagonia, Argentina). The study was performed on twelve strains (nine pigmented and three colourless), four of which have been recently reported as new species.[8] In this work, we analyse and report the degree of carotenoid and mycosporine synthesis induced upon experimental exposure to PAR and UVR.

Materials and methods

Study area

Several lakes, rivers, and ponds from Nahuel Huapi National Park in Northwestern Patagonia (Argentina) were surveyed for the occurrence and diversity of yeasts (Table 1). These bodies of water are located approximately around 41°05′S, 71°30′W and occur at altitudes ranging from 800 to 1700 m above sea level.

Yeast collection and isolation

Water samples were taken by manually opening a sterilised 500 ml glass bottle at ~0.2 m depth. A total of 100 yeast strains

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Table 1 Yeast strains used in experiment 1. Pigmentation developed under the different light conditions, following Rayner,[17] and response in carotenoid and mycosporine production under UVR treatment

Yeast species (strain collection number)	Taxonomic grouping[a] (life-cycle type)	Source	Pigmentation[b]			UVR induction	
			Dark	PAR	PAR + UVR	Carotenoids	Mycosporines
Rhodosporidium babjevae (CRUB 1025)	Ba, U, Sp (teleomorphic)	Lake Nahuel Huapi	+++M	+++M	+++M	–	–
Sporobolomyces sp. A (CRUB 1038)[c]	Ba, U, Sp (anamorphic)	Lake Fonck	+++P	+++P	+++S	–	–
Sporobolomyces sp. B (CRUB 1044)[c]	Ba, U, Sp (anamorphic)	Lake Fonck	+++M	+++M	+++M	–	–
Sporidiobolus salmonicolor (CRUB 1039)	Ba, U, Sp (teleomorphic)	Lake Nahuel Huapi	+P	++P	+++P	+	–
Rhodotorula minuta (CRUB 0076)	Ba, U, E[d] (anamorphic)	Lake Mascardi	+P	++P	+++P	+	+
Rhodotorula pinicola (CRUB 1028)	Ba, U, E[d] (anamorphic)	Lake Nahuel Huapi	+P	++P	+++P	+	+
Rhodotorula sp. (CRUB 1032)[c]	Ba, U, E[d] (anamorphic)	Ventisquero Negro Pond	+P	++P	+++P	+	+
Cystofilobasidium infirmominiatum (CRUB 1045)	Ba, H, C (teleomorphic)	Lake Nahuel Huapi	++P	++P	++P	+	–
Cryptococcus sp. (CRUB 1046)[c]	Ba, H, C (anamorphic)	Lake Mascardi	+++P	+++P	+++P	+	–
Cryptococcus laurentii (CRUB 0042)	Ba, H, T (anamorphic)	Lake Toncek	W	+S	+S	+	+
Torulaspora sp. (CRUB 0068)	A, Sa, Sac (teleomorphic)	Lake Toncek	W	W	—	–	–
Candida pseudointermedia (CRUB 0037)	A, Sa, Sac (anamorphic)	Lake Toncek	W	W	—	–	–

[a] Phylum, class, order. Taxonomic abbreviations: Ba = Basidiomycetes; A = Ascomycetes; U = Urediniomycetes; H = Hymenomycetes; Sa = Saccharomycetes; Sp = Sporidiobolales; E = *Erythrobasidium* clade; C = Cystofilobasidiales; T = Tremellales; Sac = Saccharomycetales. [b] Colour abbreviations: M = miniatus; S = salmoneous; P = persicinus; W = white. [c] New species. [d] Without a valid taxonomic rank.

were isolated from water samples as described by Brizzio and van Broock.[9] 'Red' (*i.e.* carotenogenic) strains were identified by molecular characterisation,[8] while 'white' (*i.e.* non-carotenogenic) strains were identified by physiological tests.[9] After isolation, cultures were maintained at 4 °C on potato dextrose agar[10] or MYP agar (yeast extract 0.5, malt extract 7, soytone 2.5, and agar 15 g l^{-1}) media. Twelve strains, belonging to different taxonomic groups (Table 1), were used in the experiments reported below.

UVR induction experiments

Three laboratory experiments were performed to assess the effect of PAR and UVR on the production of PPCs (carotenoids and UV-absorbing compounds) in the subset of twelve (nine 'red' and three 'white') yeast strains (Table 1). For the first experiment, two-day-old cultures were used to inoculate the experimental test tubes containing melted MYP agar (45 °C) cooled in slants. Quartz tubes were used for the UVR treatments and regular glass tubes, shielded with Ultraphan-395 film (Digefra UV Opak; cut-off 395 nm), for the PAR treatments and the dark controls (wrapped in aluminium foil). Each treatment was run with two identical samples. The tubes were incubated for 4 days at 18 °C inside an environmental test chamber (Sanyo MLR 350) with a 12/12 light/dark photoperiod. The chamber was illuminated with ten white light fluorescent tubes (Sanyo, 40 W) and five Q-Panel 340 fluorescent tubes, resulting in PAR, UVA, and UVB irradiances of 66, 15, and 0.7 W m^{-2}, respectively. After exposure, the cells from each tube were suspended in a known volume of distilled water. The number of cells was quantified by counting aliquots in a Neubauer chamber. The remaining material was centrifuged at 10 000 rpm for 10 min and subsequently frozen at −20 °C for further analyses.

A second experiment was carried out using the four strains that were found to produce UV-absorbing compounds in the first experiment (Table 2) with the purpose of evaluating their ability to synthesise this compound when grown in liquid culture. In addition, we expected to obtain enough biomass of the four strains to identify the UV-absorbing compounds using high performance liquid chromatography (HPLC). The strains were cultured in potato dextrose liquid media [PD; supplemented with 1 g l^{-1} $(NH_4)_2SO_4$]. Quartz test tubes containing 9 ml of PD were inoculated with 1 ml extracts from two-day-old yeast cultures. The tubes were attached to a vertically rotating (1 rpm) plankton wheel to prevent cell settling. The experimental design consisted of two identical samples exposed to PAR and UVR (PAR + UVR) and a dark control for each of the four strains. Radiation conditions were identical to those described above. After 4 days incubation under the same conditions as the first experiment, each sample was sub-sampled for dry weight measurements and analysis of UV-absorbing compounds (spectrophotometric scans of aqueous methanol extracts on fresh material and HPLC on lyophilised material, see below).

A third experiment was set up to study the dynamics of production of UV-absorbing compounds in *Rh. minuta*. Six quartz tubes, each containing 10 ml distilled water, were inoculated with 5 ml of a one-day-old *Rh. minuta* culture grown in liquid PD at 25 °C in an incubator shaker (Innova 4000). The experimental design consisted of two samples exposed to PAR + UVR and a dark control. Overall culture conditions were identical to those described for experiments 1 and 2. Two samples for each treatment were removed from the plankton wheel at 6 and 12 h after exposure. Each sample was sub-sampled for dry weight measurements and analysis of UV-absorbing compounds (as above for experiment 2).

Extraction and analysis of carotenoid pigments

The extraction of carotenoid pigments was performed following the method of Sedmark *et al.*,[11] with modifications. Frozen

Table 2 Yields of mycosporine-glutaminol-glucoside in four yeast strains exposed to PAR + UVR or darkness in experiment 2

Yeast species (strain collection number)	Mycosporine concentration ± SD/ μg mg(dry weight)$^{-1}$	
	PAR + UV	Dark
Rhodotorula minuta (CRUB 0076)	5.168 ± 3.286	0.916 ± 0.031
Rhodotorula sp. (CRUB 1032)	2.158 ± 0.076	0.001 ± 0.002
Rhodotorula pinicola (CRUB 1028)	3.690 ± 0.118	0.070 ± 0.001
Cryptococcus laurentii (CRUB 0042)	3.379 ± 1.317	0.003 ± 0.004

dried cells in 1.5 ml Eppendorf vials were treated with 500 μl of dimethyl sulfoxide (DMSO) preheated to 55 °C and the material centrifuged. The procedure was repeated twice and the resulting supernatants pooled. The remaining pigments were extracted with acetone until any perceptible colour in the pellet had completely disappeared. These supernatants were also pooled with those previously obtained. The DMSO–acetone mixture was then extracted using petroleum ether (bp 35–60 °C) after addition of an appropriate amount of a saturated solution of NaCl to ensure phase separation. Samples were protected from exposure to direct light during the procedure and, when possible, were kept on ice and flushed with N_2. The petroleum ether extracts were washed once with distilled water and the solvent evaporated under N_2 flux. The residue was suspended in a known volume of fresh petroleum ether and the pigments were measured spectrophotometrically (Genesys Spectronic 20) at 485 nm. Carotenoid concentration is expressed as μg of total carotenoids per 10^{10} cells using the extinction coefficient of torulene (2680 dm^3 mol^{-1} cm^{-1}, taken from Simpson *et al.*[12]).[13]

Extraction and analysis of UV-absorbing compounds

In experiment 1, we performed a spectrophotometric analysis to detect the presence of UV-absorbing compounds and determine their concentration in the twelve yeast strains. In experiments 2 and 3, UV-absorbing compounds were identified and their concentrations assessed by HPLC of lyophilised samples sent to the University of Innsbruck, Austria. Spectrophotometric determinations were performed on 20% methanol extracts (24 h at 4 °C, followed by 2 h at 45 °C) following Sommaruga and Garcia-Pichel[14] and Laurion *et al.*[15] The extracts obtained after centrifugation and filtration through GF/F filters were scanned in a Hewlett Packard P 8453-E UV-Vis spectrophotometer using 1 cm quartz Suprasil cuvettes.

For HPLC analysis, freeze-dried yeast samples were extracted three times consecutively in 25% aqueous methanol (v/v) for 2 h in a water bath at 45 °C. At the beginning of the first extraction, samples were placed on ice and treated with a tip sonicator (diameter 2 mm) for 1 min at 0.5 cycles and 20% amplitude (Hielscher UP 200S). The extracts were then cleared by centrifugation at 16000*g* and stored at −80 °C for further characterisation using HPLC. For separation and quantification of the UV-absorbing compounds, 20 μl aliquots were injected into a Phenomenex Phenosphere C8 column (250 × 4.6 mm, pore size 5 μm), protected with a Brownlee RP-8 guard column, for isocratic reverse-phase HPLC analysis. During the analysis, samples in the autosampler were kept at 15 °C, while the column was maintained at 20 °C. The mobile phase consisted of 0.1% acetic acid in 25% aqueous MeOH (v/v) running at a flow rate of 0.79 ml min^{-1}. The UV-absorbing compounds in the eluate were detected with a Dionex UVD340S diode array detector using four pre-selected channels (310, 320, 334, and 360 nm). Peak purity was checked by analysing the spectrum over the entire wavelength range. Mycosporine-glycine and mycosporine-taurine standards were used for co-cromatography analysis.

Mycosporine quantification in both protocols was based on the 310 nm absorbance values and the extinction coefficient of the UV-absorbing compound (25000 dm^3 mol^{-1} cm^{-1}, taken from Bouillant *et al.*[16]) and were expressed as μg per 10^{10} cells for experiment 1 or as μg mg(dry weight)$^{-1}$ in experiments 2 and 3.

Results

Colony growth

The twelve yeast strains used in our experiments developed well when exposed to PAR and darkness; however, we observed different growth patterns among strains upon PAR + UVR treatment. The 'red' strains, *Rh. babjevae*, *Sporobolomyces* sp. A, *Sporobolomyces* sp. B, *S. salmonicolor*, *Rh. minuta*, *Rh. pinicola*, and *Rhodotorula* sp., grew similarly under the PAR + UVR and the other treatments. The 'white' strains *Candida pseudointermedia* and *Torulaspora* sp. were inhibited by UVR, as suggested by the poor growth observed during PAR + UVR treatment. In contrast, the third 'white' yeast, *C. laurentii*, developed successfully under PAR + UVR treatment.

After exposure, some of the strains showed changes in pigmentation that varied according to the treatment. Colour changes were recorded visually using the colour-scale table of Rayner[17] with the addition of a quantitative factor (+, −) to enhance its precision. Some 'red' yeasts showed little, if any, differences in pigmentation between the treatments, while others increased their colouration noticeably after PAR and PAR + UVR treatment (Table 1). *C. laurentii*, although regarded as non-carotenogenic, presented a slight pigmentation when exposed to PAR or PAR + UVR (Table 1).

Carotenoid pigments

Total carotenoid production and its stimulation by PAR or PAR + UVR exposure varied within the group of 'red' strains studied. The basal level of carotenoids (or constitutive carotenoid content) observed in the different coloured strains after incubation in darkness presented values ranging between 3 and 27 μg total carotenoid per 10^{10} cells [Fig. 1(a)]. Carotenoid production was strongly stimulated in *S. salmonicolor*, *Rh. minuta*, *Rh. pinicola*, and *Rhodotorula* sp. after both PAR and PAR + UVR treatments as compared to the control. In these strains, the increase in mean total carotenoid content ranged from 6 to 25% after PAR treatment and from 170 to 800% after PAR + UVR treatment compared to the basal carotenoid content (assigned as 100%). The 'red' strains *S. salmonicolor*, *Rh. minuta*, *Rh. pinicola*, *Rhodotorula* sp., *C. infirmominiatum* and *Cryptococcus* sp. produced more total carotenoids after PAR + UVR than PAR treatment. *Rh. babjevae* and *Sporobolomyces* sp. B showed a similar response to the three treatments while *Sporobolomyces* sp. A presented a higher total carotenoid content after the PAR treatment [Fig. 1(a)]. Interestingly, the colourless strain *C. laurentii*, which did not synthesise carotenoids in dark conditions, produced small amounts of these pigments during the PAR and PAR + UVR treatments.

UV-absorbing compounds

The results of the first experiment showed that only four of the twelve yeast strains tested, the 'red' yeasts *Rh. minuta, Rh. pinicola*, and *Rhodotorula* sp. and the colourless *C. laurentii*

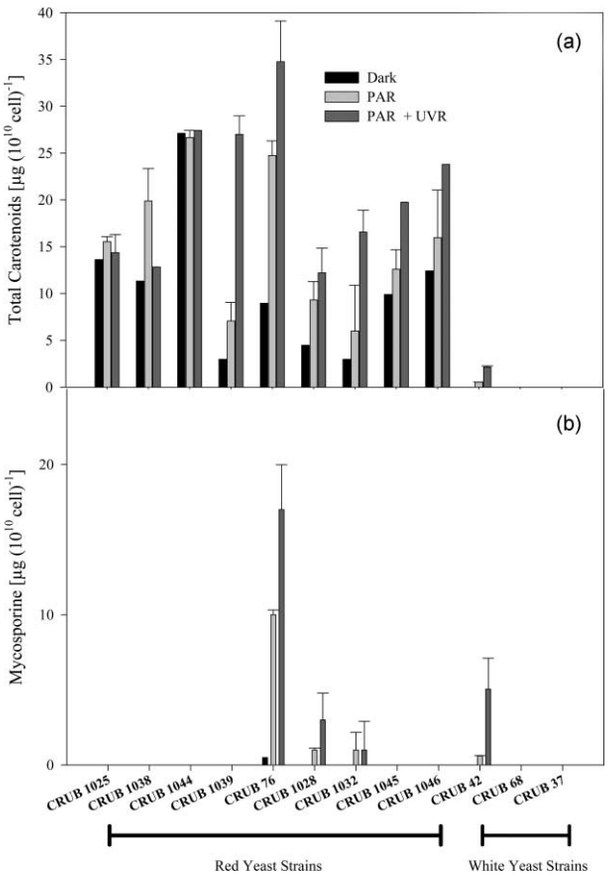

Fig. 1 Total carotenoids (a) and mycosporine (b) production (µg per 10^{10} cells) presented by wild yeast strains after incubation under different light conditions.

produced a UV-absorbing (309–310 nm) compound when exposed to PAR or PAR + UVR [Fig. 1(b) and 2]. The results of experiment 2 confirmed that under PAR + UVR treatment, the synthesis of UV-absorbing compounds was highly stimulated in the former. HPLC analysis of samples from experiment 2 showed the presence of a major compound with an absorption maximum at 310 nm, which explains the peaks observed at 309–310 nm in experiment 1 (Fig. 3) and 2. This compound did not elute together with either mycosporine-taurine (absorption maximum 309 nm) nor with mycosporine-glycine (absorption maximum 310 nm). Further characterisation by LC/MS/MS identified this compound as mycosporine-glutaminol-glucoside. The molecular structure of mycosporine-glutaminol-glucoside (myc–glu–glu, $C_{19}H_{32}N_2O_{11}$, molecular weight 484) has been reported elsewhere.[24] *Rh. minuta* was the only strain that contained any constitutive mycosporine (0.5 µg per 10^{10} cells), as was observed following the dark treatment in experiment 1. In this strain, the production of myc–glu–glu in experiment 1 increased 20-fold with PAR exposure and 34-fold upon PAR + UVR treatment compared to the control. The rest of the yeast strains, which did not synthesise mycosporines in darkness, were found to produce myc–glu–glu in concentrations up to 3 times higher upon PAR + UVR treatment compared to PAR alone [Fig. 1(b)].

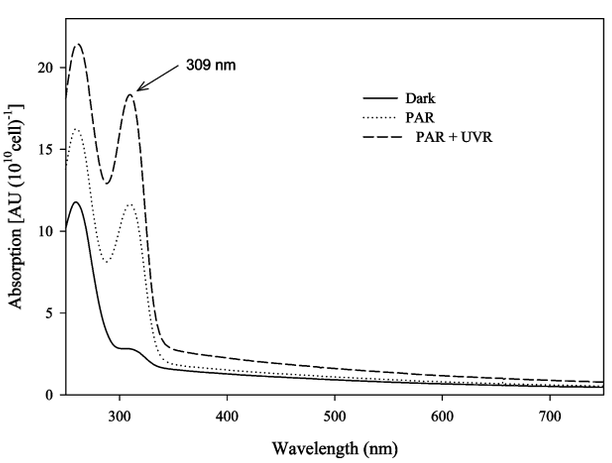

Fig. 2 Absorption spectra of methanolic extracts of *Rh. minuta* (normalised to cell concentration) grown under different light conditions.

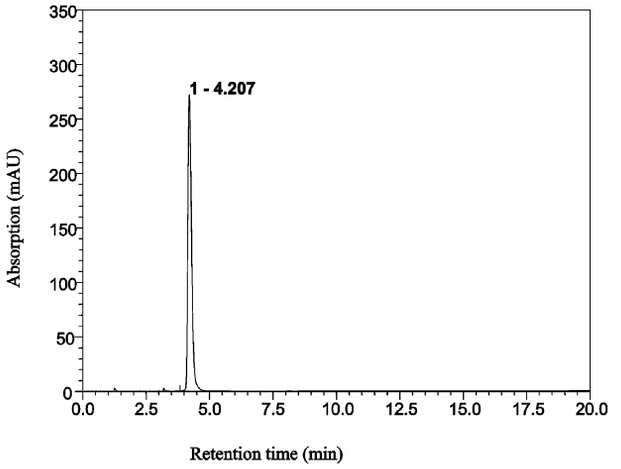

Fig. 3 HPLC chromatogram from an aqueous methanolic extract of *Rh. minuta* after PAR + UVR exposure showing the retention time of mycosporine-glutaminol-glucoside. Mobile phase: 0.1% acetic acid in 25% aqueous MeOH (v/v); flow rate: 0.79 ml min^{-1}.

The results of experiment 3 showed that the synthesis of the myc–glu–glu in exponentially growing cells of *Rh. minuta* starts within the first 6 h of exposure to PAR + UVR. In this strain, we observed that from an initial concentration of myc–glu–glu of ~1.3 µg mg(dry weight)$^{-1}$, there was a twofold increase in the first 6 h and almost fivefold after 12 h of exposure, when the concentration reached 7 µg mg(dry weight)$^{-1}$ (Fig. 4). After 12 h in darkness, the myc–glu–glu concentration for *Rh. minuta* was found to be similar to the initial value.

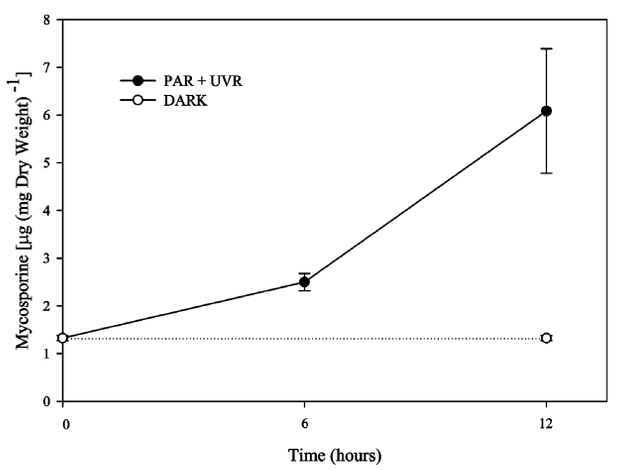

Fig. 4 Mycosporine content in *Rh. minuta* after incubation in darkness and under PAR + UVR irradiation for 6 and 12 h. Values are means ± SD.

Relationship between basal carotenoid content and induced carotenoid production

Total carotenoid production after the PAR + UVR treatment (as percentage of basal carotenoid concentration) was found to be significantly related to the basal concentration of carotenoids ($R^2 = 0.885$, $P = 0.0015$). Total carotenoid production decreased exponentially with increasing concentration of basal carotenoids (Fig. 5). The adjusted exponential model has the form TC = 99.2 + $(1178.78)^{-1.64BC}$, where TC is the total carotenoid

production and BC is the basal carotenoid content. It appears that there are two different patterns in the group of carotenogenic strains studied. The synthesis of carotenoids in those strains presenting low (<2 μg per 10^{10}cells) basal concentrations of these pigments is rapidly induced by UVR-PAR stress. In contrast, strains presenting higher basal carotenoid concentrations appear to be more resilient to the effect of PAR + UVR exposure.

Relationship between total carotenoids and mycosporine production

The production of myc–glu–glu was found to increase with increasing carotenoid synthesis in the 'red' yeasts *Rh. minuta*, *Rh. pinicola*, and *Rhodotorula* sp. after exposure to PAR and PAR + UVR. Interestingly, the same pattern was observed for the colourless *C. laurentii* (Fig. 6).

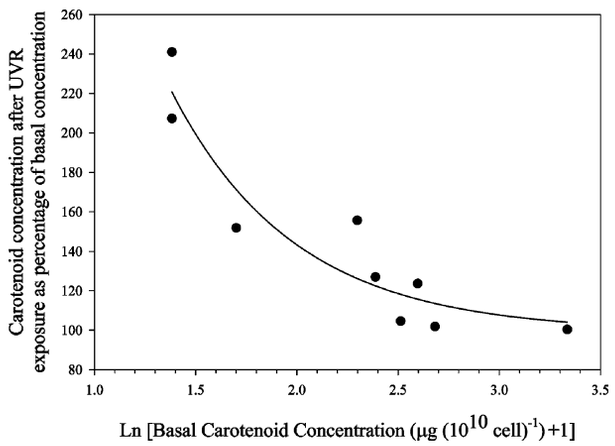

Fig. 5 Relationship between basal carotenoid content, expressed as ln(basal carotenoid concentration + 1), and the increase in total carotenoids in the 'red' wild yeast strains exposed to different light conditions.

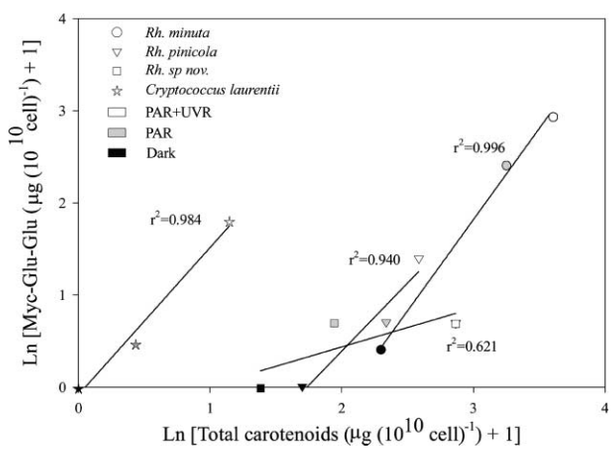

Fig. 6 Production of myc–glu–glu as a function of total carotenoid content in four wild yeast strains exposed to different light conditions.

Taxonomic trends in mycosporine production

All the yeast strains belonging to the Phylum Basidiomycota produced carotenoids either constitutively or by induction by PAR or PAR + UVR radiation (Table 1). In addition to these, three strains included in the *Erythrobasidium* clade (Class Urediniomycetes) and one belonging to the Order Tremellales also produced myc–glu–glu (Tables 1 and 2). The two strains belonging to the Phylum Ascomycota did not synthesise either carotenoids or mycosporines (Table 1).

Discussion

The twelve yeast strains assayed in the present study developed in complete darkness as well as in the presence of PAR. All the 'red' yeasts (nine strains) also developed in the presence of PAR + UVR. In contrast, only one out of three 'white' yeast strains developed under PAR + UVR. In the cultures grown in darkness, the presence of carotenoids was only detected in the nine 'red' strains [Fig. 1(a), Table 1]. Two 'red' strains (*Rh. minuta* and *Rh. pinicola*) also produced trace amounts of mycosporine, even when grown in darkness [Fig. 1(b), Tables 1 and 2]. The presence of PAR stimulated the production of carotenoids and of mycosporine in some but not all 'red' strains. Interestingly, PAR also stimulated the production of both carotenoids and mycosporine in the only 'white' strain that developed under PAR + UVR exposure. In other words, all strains that developed under PAR + UVR were able to synthesise carotenoids either constitutively or in response to PAR exposure, and a few of them also produced myc–glu–glu when exposed to PAR. Collectively these results suggest that the presence of carotenoids, either alone or in combination with mycosporines, are required for sustaining growth under exposure to PAR + UVR.

The synthesis of carotenoid compounds was clearly stimulated in six out of nine 'red' strains upon exposure to PAR alone or PAR + UVR . In general, the latter treatment produced a stronger response than PAR alone [Fig. 1(a)]. 'Red' yeast species usually accumulate β-carotene, torulene, and torularhodine as their major carotenoid pigments.[3–5,12] Previous results with a *Rh. minuta* strain suggested that no changes in these pigments composition were observed when the yeast was exposed to UVR.[18] With the exception of *Sporobolomyces* sp. A, we did not observe any noticeable change in the colour of colonies after the experiments (Table 1).

Within the set of 'red' strains, the increase in carotenoid concentration under PAR + UVR exposure (expressed as a percentage of its concentration after incubation in darkness) showed a negative exponential relationship (Fig. 5). These results can be interpreted as an indication that those cells with higher constitutive levels of carotenoids are less responsive (if at all) to induction by PAR + UVR, probably because they already bear sufficient amounts of these photoprotective compounds. The reason why some strains maintain high carotenoid concentrations, regardless of the history of exposure, while others show higher plasticity (*i.e.* respond to PAR or UVR-PAR induction), remains as yet unknown.

The stimulation of the carotenoid synthesis upon exposure to PAR has previously been reported for a strain of *Rh. minuta*.[18] Also, it has been shown that short-term UVB irradiation may induce absorbance changes in the cell wall and membrane,[19] as well as potassium efflux from the cell.[20] Another report showed that a mutant strain of *Rh. glutinis* produced higher carotenoid yields when cells in stationary phase were exposed to PAR.[21] The cultures grown in darkness had very low or undetectable concentrations of mycosporines, but exposure to PAR, and particularly PAR + UVR, stimulated the production of mycosporine-glutaminol-glucoside in four strains. Within the four mycosporine-producing strains, exposure to PAR or PAR + UVR resulted in elevated concentrations of both carotenoids and mycosporines. Although the trend of the response was similar in the four strains, the relationship between carotenoid and mycosporine production was found to be significant only for *Rh. minuta* (Fig. 6). The molecular mechanisms triggering and controlling the synthesis of mycosporines and carotenoids are presently unknown; however, we cannot exclude the possibility that a common receptor, or even a common intermediary pathway, may be involved in both induction and synthesis.

All strains belonging to Basidiomycetes produced carotenoids, either constitutively (nine strains) or after PAR or PAR + UVR induction (one strain). On the other hand, mycosporines were synthesised by only four of these strains and mycosporine production was significantly induced by exposure to PAR or PAR + UVR. In contrast, the two strains belonging to Ascomycetes did not show production of any of these

pigments. Our next studies will focus on the screening of a wider group of yeast species to verify the taxonomic trends that are suggested by the results of this work.

This is the first report on the presence of mycosporines in yeasts. Mycosporine-glutaminol-glucoside has been previously identified in terrestrial Ascomycetes,[16,22–23] but, to the best of our knowledge, never in basidiomycetous fungi. Recently, Volkmann *et al*.[24] showed that myc–glu–glu, together with mycosporine-glutamicol-glucoside, are constitutive and natural secondary metabolites of ascomycetous microcolonial fungi. Fungal mycosporines, always associated with sporulating mycelia, have been assigned a photoprotective function only in a few instances.[25] In our study, neither those yeasts producing true mycelia (*i.e*. *Rh. babjevae*), nor those that sporulate (*i.e*. *Sporobolomyces* sp. A and B and *S. salmonicolor*) were able to synthesise myc–glu–glu.

We report for the first time the occurrence of mycosporine synthesis induction in an heterotrophic organism isolated from water samples. The synthesis of mycosporines by heterotrophs suggests that their biosynthesis is independent from the photosynthetic regulatory mechanisms. This result is interesting, not only because of its novelty, but also because it presents a wide number of possibilities for future research. The fact that yeasts are widely used as food supply or dietary supplements in aquaculture[26] and that by manipulating the exposure conditions of the cultures it is possible to induce or restrain the synthesis of PPCs, may contribute to the study of the mechanisms of bio-accumulation and food selection by primary consumers.

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