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# Mycosporines in carotenogenic yeasts

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## Abstract

The ability to produce mycosporines (MYCs) in 157 pigmented yeast strains (eight genera, 25 species) isolated from natural environments of Patagonia (Argentina) was assessed. The strains belong to four taxonomic groups: the Sporidiobolales and *Erythrobasidium* clade of the class Urediniomycetes, and Cystofilobasidiales and Tremellales of the class Hymenomycetes. Induction of MYCs did not occur in all yeast strains tested and appeared to be an exclusive trait of members of the *Erythrobasidium* clade and Tremellales. This is the first report on the production of MYCs by pigmented species from the latter group, as well as the first extensive screening of mycosporinogenic yeasts. The consistent occurrence of MYCs in some specific phylogenetic groups suggests this trait bears evolutionary significance and that the presence/absence of MYCs may have potential applications in yeast systematics.


***Keywords:*** Biodiversity; Carotenoids; Mycosporines; Mycosporine-glutaminol-glucoside; Photoprotective compounds; Systematics; Yeasts

## Introduction

Many yeast species accumulate carotenoids, such as $\beta$ and $\gamma$-carotene, torulene, and thorularodin [17]. The majority of these pigmented yeast species belong to the basidiomycetous classes: Urediniomycetes and Hymenomycetes. Within the Urediniomycetes class, the *Rhodosporidium/Rhodotorula glutinis* clade (Sporidiobolales order) and the *Erythrobasidium/Rhodotorula minuta* clade comprise most of the pigmented species. On the other hand, the Cystofilobasidiales and Tremellales orders include many of the carotenogenic species belonging to the Hymenomycetes class. The classification of pigmented yeast isolates into the former four clades requires the application of extensive morphological, biochemical, and physiological tests or the use of rDNA sequencing.

The synthesis of carotenoids (antioxidants) and/or mycosporines (sunscreens) is a well-known strategy for photoprotection in many organisms [14]. Mycosporines, are water soluble UV-absorbing (310–320 nm) compounds containing an aminocyclohexenone unit bound to an amino acid or amino alcohol group [2]. Mycosporines (MYCs) differ from the so-called mycosporine-like aminoacids (MAAs) in that the latter have preferentially an

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aminocyclohexenimine unit and in that they are only found in marine and freshwater organisms [2,15].

Although mycosporines were initially discovered in fungal sporulating mycelia [7,20], it was not until recently that their synthesis was reported in yeasts by Libkind et al. [11] who found that a number of basidiomycetous carotenogenic yeasts synthesized a UV-absorbing compound (peak absorption at 309–310 nm) when grown under photosynthetically active radiation (PAR, 400–750 nm). In a subsequent study, the compound was identified as mycosporine-glutaminol-glucoside [19].

Patagonian natural habitats harbour a large number of yeast species belonging to the four previous taxonomic groups, including a few species not yet described [8,10]. Thus, they appear as a biodiversity rich area deserving further scrutiny. Formerly, we had surveyed the biodiversity of carotenoid producing yeasts from natural habitats in Patagonia [4,8]. Isolates collected during this survey were identified using a battery of methods including conventional techniques and molecular biology methods, such as PCR fingerprinting and sequencing of the D1/D2 domains of the 26S rDNA [8]. In this paper, we used a large set of accurately identified carotenogenic yeast strains and species, to investigate the relationship between taxonomic position and the synthesis of mycosporine-glutaminol-glucoside.

## Materials and methods

### Isolation and identification of yeast strains

The 157 strains included in this study were collected from natural environments of Northwestern Patagonia. They were isolated mostly from aquatic habitats but, some strains from terrestrial substrates such as soil, nectar, wild fruits and *Cyttaria* sp. fungus were also included [4,8]. The strains identification was performed employing a polyphasic approach combining conventional and molecular techniques as described in Libkind et al. [8]. Conventional tests included the detection of ballistospores and teliospores [8], the assimilation of inositol and D-glucuronate as sole carbon sources; the assimilation of nitrate as sole nitrogen source, and the production of amyloid compounds (PAC) [22]. Final identification was achieved by grouping cospecific strains with the micro/minisatellite primed PCR technique and then employing 26S rDNA partial sequence analysis to at least one representative strain of each group [8].

### Mycosporine induction

Synthesis of mycosporines was induced by transferring young cultures (24 h) to YPD agar medium ($g\,L^{-1}$): yeast extract 10, peptone 20, glucose 20, agar 15 for screening purposes or to MMS solid medium ($g\,L^{-1}$): glucose, 10; $(NH_4)_2SO_4$, 2; $KH_2(PO_4)$, 2; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.1; Yeast extract, 1, pH 5, for quantitative purposes. The plates were incubated 4 days at 18 °C in an environmental test chamber (SANYO MLR 350) with a 12:12 light:dark photoperiod. The chamber was illuminated with 10 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. For the screening analysis, Petri dishes containing isolated colonies were shielded with Ultraphan-395 film (UV Opak, Digefra, Munich, Germany, cut-off: 395 nm) and exposed only to PAR. Plates containing mycosporinogenic strains were covered with quartz glass and exposed to PAR + UVR for quantitative studies. After exposure, the colonies were transferred to distilled water, centrifuged and conserved at −20 °C until mycosporine extraction. Each test was run in triplicate.

### Mycosporine extraction and analysis

Samples were extracted with 1 ml of a methanolic aqueous solution (20%), vigorously agitated in a vortex mixer, incubated 2 h at 45 °C and centrifuged [18]. The resulting supernatant was diluted with the methanol solution and used as such in spectrophotometric analysis at 310 nm, in a Hewlett Packard P 8453-E UV–Vis spectrophotometer using 1-cm quartz Suprasil cuvettes. Spectrophotometric analysis of the sample with or without previous filtration through GF/F filters gave identical qualitative and quantitative results. HPLC analysis was performed as reported in Libkind et al. [11] over extracts of MYC positive species and representative strains of each MYC-negative species for confirmation. MYCs quantification was based on the 310 nm absorbance values and the molar extinction coefficient of MGG (25,000 $M^{-1}\,cm^{-1}$, according to Bouillant et al. [3]). Data was expressed as mg of MGG per g of dry weight (d.w.). Dry biomass was calculated as previously described [9], by drying samples until constant weight at 95 °C.

## Results and discussion

The 157 basidiomycetous yeast strains belonging to eight genera and 25 species were assigned to four taxonomic groups that comprised the majority of the known pigmented yeast species: the Sporidiobolales and *Erythrobasidium* clade of the class Urediniomycetes, and Cystofilobasidiales and Tremellales of the class Hymenomycetes. Seven undescribed yeast species classified in the genera *Cryptococcus* (two species), *Dioszegia*,

*Cystofilobasidium*, *Rhodotorula* (two species), *Sporobolomyces* and *Sporidiobolus*, were also studied (Table 1).

The observation of a maximum absorption peak at 310 nm in spectrophotometric analysis of the methanolic extracts (Fig. 1) was the indication for the presence of MYCs. These samples were afterwards qualitatively and quantitatively analyzed by HPLC and by spectrophotometry, respectively.

Not all yeast strains tested were able to synthesize MYCs (Table 1). None of the 11 species (132 strains) of the order Sporidiobolales produced MYC when stimulated with PAR or with PAR + UV (data not shown). In contrast, all five species (eight strains) included in the *Erythrobasidium* clade synthesized MYCs when grown under PAR or in some cases even constitutively [11]. Within the Class Hymenomycetes, those species belonging to the Tremellales (four species, four strains) were mycosporinogenic while members of the Cystofilobasidiales (four species, 13 strains) were not. In our previous work [11], similar taxonomic trends on the occurrence of MYCs in yeasts were shown, even though a reduced number of strains were studied. The examination of a larger number of pigmented strains presented in this work, indicates that only species belonging to the *Erythrobasidium* clade and the order Tremellales seem to be able to synthesize MYC. Besides the strains of the Tremellales analyzed here, other members of the Tremellaceae family such as the non- or cream-colored species *Cryptococcus laurentii* [11] and *Cr. victoriae* (unpublished results), are known to produce MYC.

All strains within a species produced consistent results (i.e., they were all MYC+ or MYC−). At a higher taxonomical level, all species within an order/clade responded identically. Thus, although we admit that the number of mycosporinogenic species included in this study is relatively low, the consistency of the results encourages the hypothesis that the production of MYC is a taxon-specific character.

New pigmented yeasts isolated from natural environments may be difficult to assign to any of the four taxonomic groups. A routine of often expensive, time consuming, and not completely reliable morphological,

**Table 1.** MYC production screening of basidiomycetous pigmented yeast species from Patagonia

| Species and taxonomic placement | No. of strains tested | MYC |
|---|---|---|
| CLASS UREDINIOMYCETES SPORIDIOBOLALES | | |
| *Rhodotorula mucilaginosa* | 89 | − |
| *Rhodosporidium babjevae* | 17 | − |
| *Rhodosporidium kratochvilovae* | 3 | − |
| *Rhodosporidium diobovatum* | 1 | − |
| *Rhodotorula* sp.[a] | 3 | − |
| *Rhodotorula graminis* | 1 | − |
| *Rhodotorula colostri* | 3 | − |
| *Sporidiobolus longiusculus*[a] | 3 | − |
| *Sporidiobolus salmonicolor* | 4 | − |
| *Sporobolomyces roseus* | 2 | − |
| *Sporobolomyces ruberrimus* | 4 | − |
| *Sprobolomyces patagonicus*[a] | 2 | − |
| *ERYTHROBASIDIUM CLADE* | | |
| *Rhodotorula laryngis* | 2 | + |
| *Rhodotorula minuta* | 3 | + |
| *Rhodotorula slooffiae* | 1 | + |
| *Rhodotorula* sp.[a] | 1 | + |
| *Rhodotorula pinicola* | 1 | + |
| CLASS HYMENOMYCETES CYSTOFILOBASIDIALES | | |
| *Cryptococcus macerans* | 2 | − |
| *Cystofilobasidium capitatum* | 6 | − |
| *Cystofilobasidium infirmominiatum* | 2 | − |
| *Cystofilobasidium* sp.[a] | 3 | − |
| TREMELLALES | | |
| *Dioszegia hungarica* | 1 | + |
| *Dioszegia* sp.[a] | 1 | + |
| *Cryptococcus* sp. A[a] | 1 | + |
| *Cryptococcus* sp. B[a] | 1 | + |

[a]Novel yeast species from Patagonia Argentina. CRUB, Centro Regional Universitario Bariloche Culture Collection. MYC, Mycosporine.

biochemical and physiological tests is required for identification. We found that a correct and rapid sorting of pigmented yeast species into each of the four taxonomic groups may be assessed by combining results of production of amylaceous compounds and synthesis of MYCs (Table 2), using this as a chemo-taxonomical character. However, the ability to produce MYCs of a larger number of species including type strains from culture collections is to be conducted in our laboratory to confirm this hypothesis.

Our HPLC analysis performed to identify the synthesized compound of mycosporinogenic strains revealed in almost all cases a single peak exhibiting identical retention time and maximum absorption (310 nm) as that of MGG [19]. The only exception was the strain *Cryptococcus* sp. A CRUB 1152 which showed a second minor peak (17%) yet unidentified (Fig. 2).

Quantitative studies on MYC synthesis showed variability among the different species and strains tested (Table 2). MYC production reached high values ranging from 13 to 48 mg $g^{-1}$ d.w. The two strains belonging to the *Dioszegia* genus produced significantly higher amounts ($P<0.01$) than other species and concentrations registered were higher than those reported previously [11] (Table 3).

In contrast to carotenoids, mycosporines and specially MGG are not known to play a photoprotective

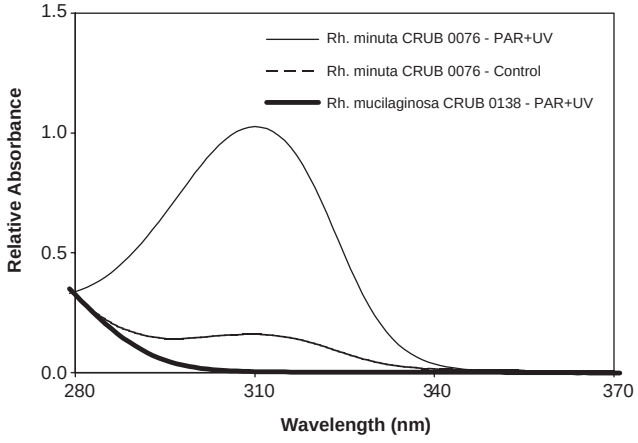


**Fig. 1.** Absorbance spectra of methanolic yeast extracts from a MYC positive (*Rhodotorula minuta*) and MYC negative (*R. mucilaginosa*) species under different light conditions.

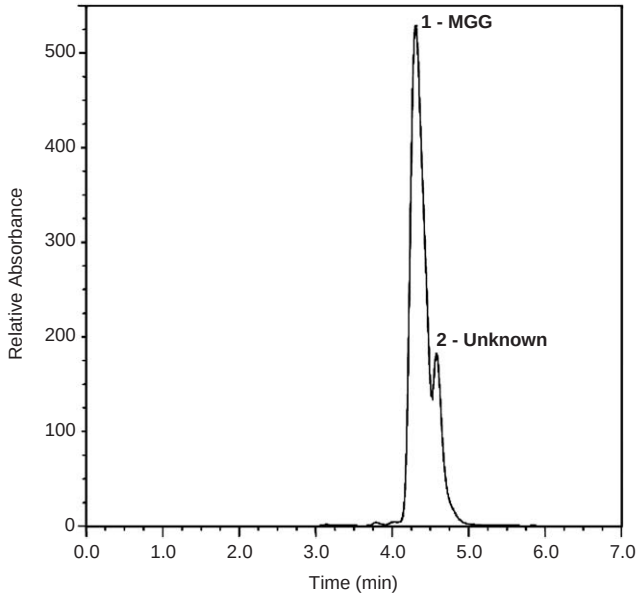


**Fig. 2.** HPLC chromatogram of *Cryptococcus* sp. A CRUB 1152 detected at 310 nm.

**Table 2.** Salient morphological, physiological, and biochemical characteristics for the four taxonomic groups, based on the pigmented species studied

| Characteristics | Urediniomycetes | | Hymenomycetes | | |
|---|---|---|---|---|---|
| | Sporid. | *Eryth.* Clade | Cystof. | Trem. | Time[a] (weeks) |
| Ballistospores[b] | *V* | –[c] | – | *V* | 1 |
| Teliospores[b] | *V* | –[c] | *V* | – | 1–2 |
| myo-inositol[d] | *V* | – | +[c] | – | 3 |
| D-glucuronate[d] | –[c] | +[c] | + | + | 3 |
| Nitrate[e] | *V* | *V* | +[c] | – | 2 |
| PAC[f] | – | – | + | + | <1 |
| Mycosporines | – | + | – | + | <1 |

Abreviations: Sporid., Sporidiobolales; *Eryth. Erythrobasidium*; Cystof., Cystofilobasidiales; Trem., Tremellales.
[a]Procedure duration.
[b]Morphological tests.
[c]Has variable results if all known pigmented species of the taxonomic group are included in the analysis.
[d]Carbon assimilation tests.
[e]Nitrogen assimilation tests.
[f]Production of amilaceous compounds. +, positive; –, negative; *V*, variable results.

**Table 3.** Quantitative and qualitative analysis of MYC production by Patagonian native yeasts

| Species and taxonomic placement | CRUB Number | Origin | MYC production mg $g^{-1}$ d.w. | No. of MYC produced |
|---|---|---|---|---|
| ERYTHROBASIDIUM CLADE | | | | |
| *Rhodotorula minuta* | 0025 | Lake Mascardi | 25±1.3 | 1 |
| *Rhodotorula minuta* | 0076 | Lake Mascardi | 23±0.5 | 1 |
| *Rhodotorula pinicola* | 1028 | Lake Nahuel Huapi | 16.2±0.8 | 1 |
| *Rhodotorula slooffiae* | 1029 | Lake Nahuel Huapi | 20.5±0.6 | 1 |
| *Rhodotorula* sp.[a] | 1032 | Manso glacial lagoon | 12.9±0.5 | 1 |
| *Rhodotorula laryngis* | 1105 | Lake Ilon | 21.7±0.5 | 1 |
| *Rhodotorula minuta* | 1136 | Lake Negra | 32.5±1.3 | 1 |
| *Rhodotorula laryngis* | 1183 | Manso glacial lagoon | 25.4±0.4 | 1 |
| TREMELLALES | | | | |
| *Dioszegia* sp.[a] | 1147 | Lake Toncek | 47.8±0.4 | 1 |
| *Dioszegia hungarica* | 1148 | Lake Ilon | 38.8±0.6 | 1 |
| *Cryptococcus* sp. *A*[a] | 1150 | Lake Ilon | 17.8±0.3 | 2 |
| *Cryptococcus* sp. *B*[a] | 1152 | Lake Negra | 26±0.4 | 1 |

[a]Novel yeast species from Patagonia (Argentina). CRUB, Centro Regional Universitario Bariloche Culture Collection. MYC, Mycosporine. d.w., dry weight.

function in yeasts. In Fungi, MYCs were first detected in association with sporulation events [7,20]; more recently, they have also been suggested as photoprotectors due to their UV-screening and antioxidant properties [6,21,23]. Previous results obtained in our laboratory indicated that both mycosporines and carotenoids are important for sustaining yeast growth and survival under high UVR conditions [11,12].

In the present report, among 157 yeast strains from natural habitats of which many were oligotrophic aquatic lakes, only 12 strains (nine out of 25 species) were able to synthesize MYCs. However, 10 out of the 12 mycosporinogenic strains (83.3%) were isolated from surface waters of mid-high altitude (760–1700 m.a.s.l.) lakes which receive strong UVR [13]. The lakes were pooled in two groups, and MYC values of the strains recovered in those lakes averaged (Fig. 3). Non-significant differences ($P = 0.144$) were found between mean MYC production of strains from high-altitude lakes (1450–1700 m.a.s.l.) and that of the low-altitude lakes (765–795 m.a.s.l.), 30.8±11.2 and 21.2±3.8 mg $g^{-1}$ d.w., respectively. Yeasts collected from lakes at high altitudes produced the highest MYC values.

Our results suggest that mycosporinogenesis is a character related to certain phylogenetic groups. Other authors have already reported that the presence of MYCs in fungi is restricted to a few species, since some taxonomic groups or species are unable to produce them [2,20]. Similar results in other organisms, suggested that the synthesis of closely related UV-absorbing compounds, MAAs, in symbiotic dinoflagellates [1] and in sea anemones [16] reflects phylogenetic differences among these organisms rather than environmental factors. Similarly MAAs occur in cyanobacteria but they are not widespread [5].

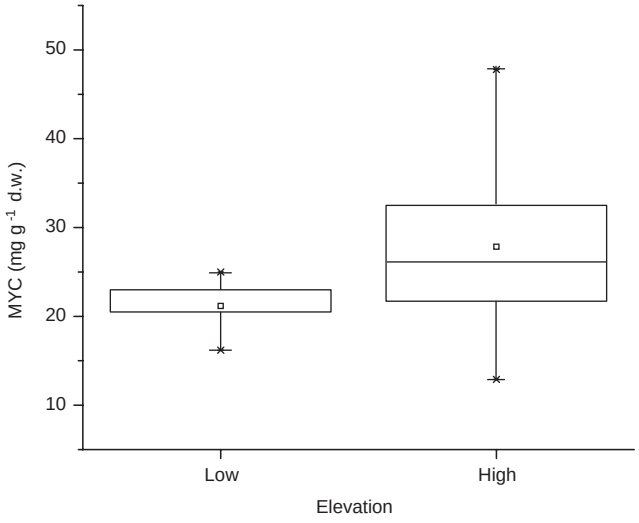


**Fig. 3.** Relationship between altitude of the lake of origin and yeast MYC production. Error bars: standard deviation.

The consistent occurrence of MYC in some phylogenetic lineages of yeasts suggests that this trait was already present in the group common ancestor. Those MGG negative yeast groups may have lost this function during evolution. MGG presence in pigmented basidiomycetous yeasts seem to be related to environmental factors and may have evolutionary significance.

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