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Fatty acid composition of cold-adapted carotenogenic basidiomycetous yeasts

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ABSTRACT

We studied fatty acids (FAs) profiles in six carotenoid-producing yeast species isolated from temperate aquatic environments in Patagonia. Total FAs ranged from 2 to 15% of dry biomass. Linoleic, oleic, palmitic and α-linolenic acids were the major FAs constituents, which accounted for as much as 40%, 34%, 13% and 9% of total FAs, respectively. The proportion of each FA varied markedly depending on the taxonomic affiliation of the yeast species and on the culture media used. The high percentage of polyunsaturated fatty acids (PUFAs) found in Patagonian yeasts, in comparison to other yeasts, is indicative of their cold-adapted metabolism. Our results suggest that Patagonian yeasts may be considered an interesting source of essential PUFAs.

Key words: native yeasts, Patagonia, carotenoids, lipids, fatty acids.

RESUMEN

Composición de ácidos grasos de levaduras carotenogénicas. Se estudiaron los ácidos grasos de seis especies de levaduras productoras de carotenoides aisladas de ambientes acuáticos templados de la Patagonia. El contenido total de ácidos grasos fluctuó entre 2 y 15% de la biomasa seca. Los ácidos linoleico, oleico, palmítico y α-linolénico fueron los mayoritarios y contribuyeron en un 40%, 34%, 13% y 9% al total de ácidos grasos, respectivamente. La proporción de cada ácido graso varió de modo considerable según la filiación taxonómica de la especie y el medio de cultivo utilizado. El alto porcentaje de ácidos grasos poliinsaturados (PUFA) observado en las levaduras patagónicas, en comparación con levaduras de otros orígenes, es indicativo de un metabolismo adaptado al frío. Nuestros resultados sugieren que las levaduras patagónicas pueden ser consideradas una fuente interesante de PUFA esenciales.

Palabras clave: levaduras nativas, Patagonia, carotenoides, lípidos, ácidos grasos

The search for unconventional sources of naturally-occurring fatty acids (FAs) is of increasing interest given their well publicized nutritional, physiological and pharmacological functions. It is now possible to produce specific oils on an industrial scale, using lipid-producing microorganisms (8). Yeast species can accumulate high levels of intracellular lipids (3). Most of the lipids produced by oleaginous yeasts are long-chain FAs comparable to conventional vegetable oils (9), and may include polyunsaturated fatty acids (PUFAs). This is of significant interest because there is ample evidence that a subset of the PUFAs, termed essential fatty acids (EFAs), plays crucial physiological and nutritional roles in animals and humans (12).

Many oleaginous yeasts also accumulate carotenoid pigments, and are therefore called red yeasts. Carotenoids are generally localized in lipid droplets in these microorganisms and also in other fungi (2). Red yeasts are a diverse group of unrelated organisms (mostly *Basidiomycota*) and the majority of the known species are distributed in four taxonomic groups (Orders): the *Sporidiobolales* and *Cystobasidiales*/*Erythrobasidiales* of the *Pucciniomycotina*, and the *Cystofilobasidiales* and *Tremellales* of the *Hymenomycotina* (10).

Numerous studies have dealt with FA production by red yeasts (1, 7, 13), however, they have only investigated a limited number of red yeast species within the *Sporidiobolales*. Thus, the aim of our research was to study FA production in a set of recently isolated yeast strains from temperate aquatic environments in Patagonia. We chose representative species of the four major red yeast groups and thus FA synthesis was also discussed from a taxonomic point of view. Finally, it was also of interest to assess PUFA production by these cold-adapted yeasts.

Table 1. Yeast strains studied, taxonomic placement and other relevant information

Species	CRUB N°	Other collections[1]	Origin	Taxonomic placement	Sexuality	Other[2]
Rhodotorula mucilaginosa	0138	–	L. Toncek	*Sporidiobolales*, *Pucciniomycotina*.	ANA	Mucous
Sporidiobolus longiusculus	1044^T	PYCC 5818^T, CBS 9654^T	L. Fonck	*Sporidiobolales*, *Pucciniomycotina*.	HET (MT A1)	Mucous–Ballisto
Sporobolomyces patagonicus	1038^T	PYCC 5817^T, CBS 9657^T	L. Fonck	*Sporidiobolales*, *Pucciniomycotina*.	ANA	Ballisto
Rhodotorula minuta	0025	–	L. Mascardi	*Cystobasidiales*, *Pucciniomycotina*.	ANA	Mucous
Cystofilobasidium lacus-mascardii	1046	PYCC 5819^T, CBS 10642^T	L. Mascardi	*Cystofilobasidiales*, *Agaricotinomycotina*.	HET (MT A1)	
Dioszegia sp.	1147	–	L. Toncek	*Tremellales*, *Agaricotinomycotina*.	ANA	Ballisto

[1]PYCC: Portuguese Yeast Culture Collection, CRUB=Centro Regional Universitario Bariloche yeast culture collection, CBS= Centraalbureau voor Schimmelcultures, ANA: Anamorphic, HET: Heterothallic, MT: Mating Type, [2]morphological characteristics: Mucous: colonies characterized by the production of large quantity of mucous (polysaccharides); Ballisto: Ballistosporinogenic (production of actively projected ballistospores).

Six pigmented yeast strains, isolated from Patagonian ultra oligotrophic aquatic environments, were selected for FA composition studies (Table 1), as described in Libkind *et al.* (4). Strain identification was performed by combining conventional and molecular techniques (4). Cultures were maintained as described in Libkind *et al.* (4).

Yeasts were grown in 250 ml Erlenmeyer flasks containing 50 ml of one of the two following media: MYP (same as above but without agar) and minimal medium salts (MMS), composed of 10.0 g/l glucose, 2.0 g/l $(NH_4)_2SO_4$, 2.0 g/l KH_2PO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 0.1 g/l $CaCl_2 \cdot 2H_2O$, and 1.0 g/l yeast extract (initial pH 5). Inocula consisted of 5% v/v of 36 h cultures using the same propagation media and culture conditions. Inoculated flasks were incubated in an INNOVA 4000 rotary incubator at 21 °C and 200 rpm. After 72 h, yeast biomass was harvested by centrifugation, washed twice with distilled water and lyophilized in a HETO (Dry Winner) freeze drier. Samples were kept under a nitrogen atmosphere, at -20 °C until further processing. Cell dry mass was determined after drying at 105 °C until constant weight.

Fatty acid methyl esters (FAMEs) were obtained in a three-step process: extraction, derivatization, and quantification on a gas chromatograph (GC) following the procedures outlined in Schlechtriem *et al.* (11). FAME concentrations were quantified on a Hewlett Packard 6890 GC configured as in Schlechtriem *et al.* (11). A 37-component FAME standard (Supelco #47885-U) was used to identify and quantify (4-point calibration curves) FAMEs in the samples (unknown) i.e. by comparing their retention times to those of the FAME standard. Results were reported as µg FAME/mg dry weight fungal tissue or as proportions (% contribution of any individual FA to total measured FAs). Statistical analysis of FA values between the two culture media was carried out using the Student's t-test.

Six different *basidiomycetous* yeast species were studied, distributed among the four taxonomic groups that include the majority of known carotenogenic yeasts (Table 1), four of which corresponded to novel taxa (4). All seven species accumulated FAs when grown on the two experimental semi-synthetic media, though with considerable qualitative and quantitative differences. Total lipids, as a percentage of dry biomass, ranged from 2.2 to 14.7% in *Rhodotorula minuta* and *Sporobolomyces patagonicus*, respectively (Table 2). These values are similar to those found in the literature for closely related species (7) and yeasts in general (3). MYP stimulated FA accumulation ($p < 0.05$) in the three *Sporidiobolales* species, while members of the other 3 groups showed higher, though not statistically significant, FA percentages in MMS ($p = 0.225$).

Saturated fatty acids (SAFAs), FAs with one double bond (MUFAs) and PUFAs were detected in yeast tissues in average proportions of 16%, 34% and 50% of total FAs, respectively. Omega-3 and omega-6 PUFAs

Table 2. Fatty acid profiles of six Patagonian carotenogenic yeast species using two different culture media.

Species	N° CRUB[1]	Culture medium	Bm[2] (g/l)	Lipids %	Relative percentage				
					Σω3[3]	Σω6[4]	ΣSAFA[5]	ΣMUFA[6]	ΣPUFA[7]
Rhodotorula mucilaginosa	0138	MMS	4.27	2.79	5.8	21.4	10.4	62.4	27.2
		MYP	4.41	10.27	8.1	28.9	18.2	44.7	37.2
Sporidiobolus longiusculus	1044	MMS	4.18	5.43	11.8	27.5	9.1	51.6	39.3
		MYP	3.11	7.38	17.6	30.1	20.6	31.7	47.7
Sporobolomyces patagonicus	1038	MMS	4.57	8.38	9.6	24.4	12.6	53.4	34.0
		MYP	2.32	14.70	24.2	30.1	26.1	19.5	54.4
Rhodotorula minuta	0025	MMS	5.02	5.60	0.9	59.1	14.1	25.9	60.0
		MYP	0.61	2.22	5.0	53.1	18.5	23.5	58.1
Cystofilobasidium lacus-mascardii	1046	MMS	4.24	12.88	9.6	42.2	15.0	33.2	51.8
		MYP	3.15	4.95	13.3	55.6	17.6	13.5	68.9
Dioszegia sp.	1147	MMS	4.32	7.98	3.7	39.0	10.1	47.2	42.7
		MYP	3.02	5.04	6.9	50.4	16.4	26.3	57.3

[1]CRUB: Centro Regional Universitario Bariloche culture collection; [2]Bm: biomass yield dw·L^{-1}; [3]Σω3: sum of omega-3 fatty acids omega-3 type, [4]Σω6 :sum of omega-6 fatty acids, [5]ΣSAFA:sum of saturated fatty acids, [6]ΣMUFA:sum of monounsaturated fatty acids, [7]ΣPUFA:sum of polyunsaturated fatty acids; nd: not determined.

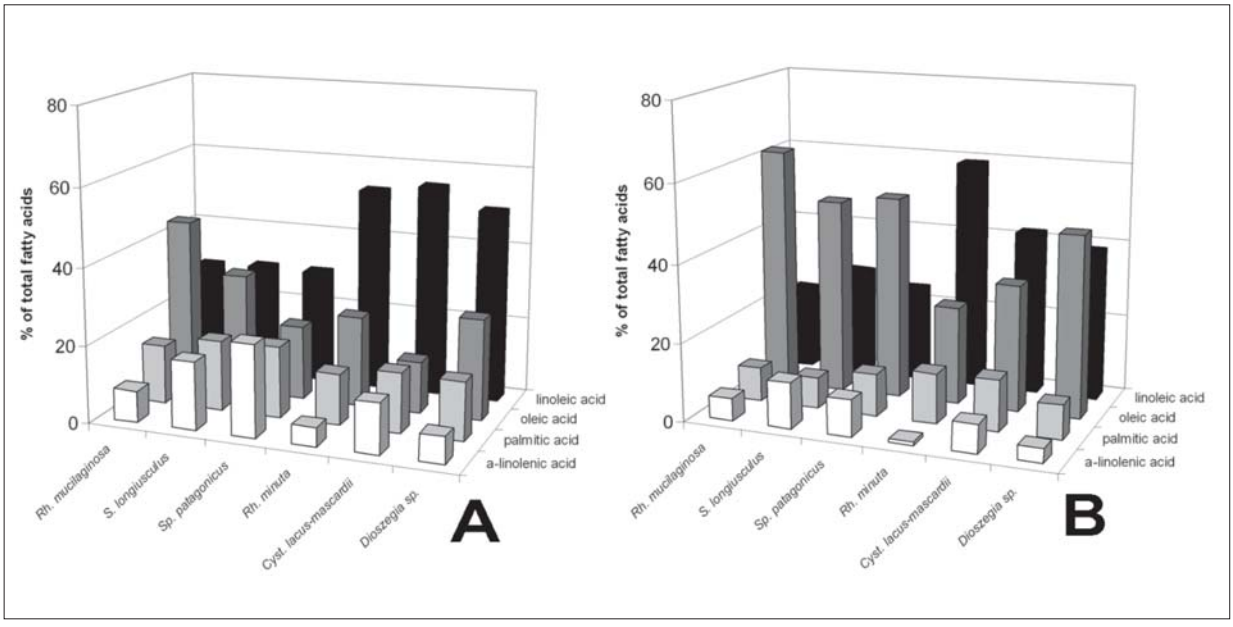

Figure 1. Relative composition of four major fatty acids found in carotenogenic yeasts.
Relative percentages of the four major fatty acids (> 5% on a dry weight basis; as a proportion of total identified FAMEs) found in carotenogenic yeasts grown in Malt Yeast extract Peptone (MYP; A) and Minimum Medium Salt (MMS; B). *Rh.*: *Rhodotorula*, *Sp.*: *Sporobolomyces*, *S.*: *Sporidiobolus*, *Cyst.*: *Cystofilobasidium*.

were accumulated in average proportions of ~9% and ~40%, respectively. We also observed an influence of culture media on SAFA, MUFA and PUFA proportions. Significant higher values of SAFAs ($p < 0.001$) were obtained when MYP was employed as culture medium. PUFA values showed also a slight increase in MYP ($p = 0.111$). MMS medium stimulated the production of MUFAs ($p = 0.023$) in all yeast species.

The analysis of FA composition in all 6 yeasts, revealed the prevalence of four major FA constituents in red yeasts: linoleic acid (18:2n-6, in an average proportion of 40% of total FA), oleic acid (18:1n-9, ~34%), palmitic acid (16:0, ~13%) and α-linolenic acid (18:3n-3, ~9%). Figures 1A and B show the distribution of these FA among red yeasts as a function of the culture media used. These results correspond to those already reported for other yeasts (3, 7, 13, 15). However, it is worth noting that Patagonian yeasts had a higher percentage of PUFAs (particularly linoleic and α-linolenic acids) in comparison to yeasts of different origin. Native strains (especially those from groups other than *Sporidiobolales*) produced in general, PUFA values > 50% of total FAs; whereas, in most yeasts, the sum of linoleic and α-linolenic acids rarely exceed 30% (7, 15). Similarly to our results, yeast species isolated from Antarctic ecosystems (13) and species considered psycrophilic (6) were reported as being enriched by PUFAs. Thus, Patagonian strains, had FA profiles typical of cold-adapted yeasts. This observation is in agreement with the low annual average temperature (< 10 °C) registered at the region of isolation. Furthermore, it can be hypothesized that intracellular carotenoids in yeasts may account for at least one of several, lipid-based, protection mechanisms against oxidative damage (14) and that red yeasts should be capable of accumulating high quantities of PUFAs. Most of the yeasts studied in the present work, showed high levels of intracellular carotenoid pigments (200-300 µg/g) (5), which, in addition to their adaptation to cold environments, is a probable explanation for the high proportion of PUFAs observed.

The production of additional FAs at trace concentrations (< 2% of total FA) was also observed. Stearic (18:0), undecaenoic (11:0), palmitoleic (16:1n7), heptadecaenoic (17:0) and elaidic (18:1n9t) acids were detected in all yeasts, while myristic (14:0), pentadecaenoic (15:0), linoleic (18:2n6c), arachidonic (20:0), γ-linolenic (18:3n6), heneicosanoeic (21:0), cis-11,14-eicosadienoic (20:2), eicosatrienoic (20:3n3), erucic (22:1n9) and nervonic (24:1n9) acids were also found in a few cases. *S. patagonicus* accumulated the broadest variety of different FAs while *Sporidiobolus longiusculus* produced the longest FAs (C22 and C24).

We observed that different species showed different behaviors depending on their phylogenetic position. For example, *Sporidiobolales* yeasts showed lower quantities of omega-6 PUFAs either in MYP ($p < 0.001$) or MMS ($p < 0.05$), and higher amounts of MUFAs and lower of PUFAs were observed in MMS broth ($p < 0.05$). These results indicate an heterogeneous response to the culture media used depending on the taxonomic affiliation of the yeast species. This information is useful for optimization purposes since not all taxonomic groups will respond similarly to changes in culture conditions. Furthermore, we speculate that *Sporidiobolales* yeasts, which are those that have been typically studied as sources of FAs, may not necessarily be the best choice for PUFA production because the other groups of carotenogenic yeasts examined here produced higher PUFA proportions and demonstrated a greater range of plasticity in their FA profiles in response to the different culture media used. However, we stress that this conclusion must be considered tentative both because the limited number of species and the relatively narrow range of culture conditions herein examined. We conclude that these Patagonian yeast species can be considered an interesting source of carotenoid pigments, PUFAs and essential lipids; and that a more thorough assessment of their potential utilization as a biotechnological supply of these compounds should be carried out.

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REFERENCES

1. Davoli P, Mierau V, Weber RW. Carotenoids and fatty acids in red yeasts *Sporobolomyces roseus* and *Rhodotorula glutinis*. Prikl Biokhim Mikrobiol 2004; 40: 460-5.
2. Davoli P, Weber RWS. Carotenoid pigments from the red mirror yeast *Sporobolomyces roseus*. Mycologist 2002; 16: 102-8.
3. Kaneko H, Hosohara M, Tanaka M, Itoh T. Lipid composition of 30 species of yeast. Lipids 1976; 11: 837-44.
4. Libkind D, Brizzio S, Ruffini A, Gadanho M, van Broock MR, Sampaio JP. Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. Antonie van Leeuw 2003; 84: 313-22.
5. Libkind D, van Broock MR. Biomass and carotenoid pigments production by Patagonian native yeasts. World J Microbiol Biotechnol 2006; 22: 687-92.
6. McMurrough I, Rose AH. Effects of temperature variation on the fatty acid composition of a psycrophilic *Candida* species. J Bacteriol 1973; 114: 451-2.
7. Perrier V, Dubreucq E, Galzy P. Fatty acid and carotenoid composition of *Rhodotorula* strains. Arch Microbiol 1995; 164: 173-9.
8. Radwan SS. Sources of C20-polyunsaturated fatty acids for biotechnological use. Appl Microbiol Biotechnol 1991; 35: 421-30.
9. Rattray JBM. Yeasts. In: Ratledge C, Wilkinson SG, editors. Microbial lipids. London/New York, Academic press, 1979, p. 555-697.
10. Sampaio JP. Diversity, phylogeny and classification of basidiomycetous yeasts. In: Agerer R, Blanz P, Piepenbring M, editors. Frontiers in Basidiomycete Mycology. IHW-Verlag, Eching, 2004, p. 49-80.
11. Schlechtriem C, Arts MT, Zellmer ID. Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting *Daphnia pulex* (Crustacea, Cladocera). Lipids 2006; 41: 397-400.

12. Siddiqui RA, Shaikh SR, Sech LA, Yount HR, Stillwell W, Zaloga GP. Omega 3-fatty acids: health benefits and cellular mechanisms of action. Mini Rev Med Chem 2004; 4: 859-71.
13. Thomas-Hall S, Watson K, Scorzetti G. *Cryptococcus statzelliae* sp. nov. and three novel strains of *Cryptococcus victoriae*, yeasts isolated from Antarctic soils. Int J Syst Evol Microbiol 2002; 52: 2303-8.
14. Woodall AA, Britton G, Jackson MJ. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship between carotenoid structure and protective ability. Biochim Biophys Acta 1997; 1336: 575-86.
15. Zlatanov M, Pavlova K, Grigorova D. Lipid composition of some yeast strains from Livingston Island, Antarctica. Folia Microbiol 2001; 46: 402-6.

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