

Up-regulated expression of desaturase genes of *Mucor rouxii* in response to low temperature associates with pre-existing cellular fatty acid constituents

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Abstract Transcriptional response of desaturase genes to low temperature was investigated in the dimorphic fungus *Mucor rouxii*. The two morphological forms of *M. rouxii*, yeast-like and mycelial cells containing different fatty acid profiles were shifted from 30 to 10°C. Both cultures exhibited significantly altered fatty acid composition, whose content in polyunsaturated fatty acids increased as consequence of the temperature shift and was accompanied by a reduction of C18:1 Δ^9 about 2 h after the temperature shift. These changes were particularly significant in phosphatidylcholine and phosphatidylethanolamine fractions. Moreover, the fatty acid profiles of monoacylglycerol and diacylglycerol were also modulated in response to the lower temperature of incubation. The changes of membrane lipids of *M. rouxii* were due to the cold-induced

expression of Δ^9 -, Δ^{12} - and Δ^6 -desaturase genes. Although the mRNA levels of the three desaturases were transiently induced by lowering the temperature, the pre-existing composition of fatty acid profiles of mycelial and yeast-like forms of *M. rouxii* may have lead to different expression profiles of desaturase genes that modified their membrane physical state under cold shock. While expression of Δ^{12} -desaturase gene contributed mainly to cold acclimation of mycelia, Δ^9 -desaturase expression was the main transcript identified in the yeast-like culture after temperature shift.

Keywords *Mucor rouxii* · Desaturase · Cell morphology · Low temperature · Gene expression

Introduction

It has been shown that membranes of poikilothermic organisms and plants are the targets of cell adaptation to temperature variation [1–3]. A special consideration has been given to the role of cellular fatty acid and lipid metabolites during acclimation [4, 5]. It has been shown that modulations of fatty acid composition in lipid membrane, especially the degree of unsaturation of fatty acyl chains during temperature change occurs through the control of fatty acid desaturation both at the level of transcription and post-transcriptional regulation [6–9]. The change in unsaturation is achieved by regulating the level of desaturation during temperature shifts. Remodeling of membrane composition and functionality is widely accepted and termed homeoviscous adaptation so to maintain proper cellular function [10, 11]. Although living cells render the similar adaptive mechanisms to temperature change, the different responses that depend on strain and temperature treatment have been reported [4].

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Unlike most microorganisms, oleaginous fungi not only contain fatty acids in phospholipids (PL) membranes, but accumulate substantial amounts of fatty acids in a distinct lipid pool (neutral lipids—NL), particularly triacylglycerol (TAG). Fatty acid composition and lipid content of these fungi depend on nutritional factors, temperature regime, oxygen concentration and pH [12–14]. Several oleaginous fungi have been studied for their enriched oil content that represents nutritionally important polyunsaturated fatty acids (PUFAs) [15]. Our previous study showed that, following a shift to a lower temperature, fatty acid composition was altered in *Mucor rouxii*, an oleaginous Mucorale strain, that synthesizes γ -linolenic acid (GLA, C18:3 $\Delta^{6,9,12}$). Further, we showed that *M. rouxii* Δ^9 -desaturase mRNA, which is responsible for the formation of monounsaturated fatty acids, was induced by the temperature downshift [16]. In addition, we previously reported changes of fatty acid composition in *M. rouxii* either growing as a yeast-like or as a filamentous mycelium that depended on their respective phenethyl alcohol (PEA) and oxygen tension [17]. Fatty acid analysis of aerobically grown yeast treated with PEA showed that oleic acid (C18:1 Δ^9) increased dramatically (it constitutes about 53% of total fatty acid). On the contrary, aerobically grown mycelia of *M. rouxii* contained oleic acid, linoleic acid and GLA in similar amounts [17]. The ability to grow in different morphological forms along with the capacity to modify fatty acid composition implies that this dimorphic fungus copes with environmental conditions. Therefore, it is important to investigate how *M. rouxii* cells containing different fatty acid profiles responds to temperature changes. In an attempt to understand the molecular basis of fatty acid and lipid metabolism associated with cold response in *M. rouxii*, we performed a comparative study of the mycelial and PEA-induced yeast cultures in response to low temperature acclimation. We monitored in *M. rouxii* the expression of three membrane-bound desaturases genes that code for Δ^9 -, Δ^{12} - and Δ^6 -desaturases, and are involved in the synthesis of unsaturated fatty acids. Fatty acid and lipid metabolites were correlated to the transcriptional expression of the desaturase genes. The present study reveals that *M. rouxii* displays subtle acclimation to temperature downshift by differentially up-regulated expression of desaturase genes that may depend on pre-existing fatty acid composition.

Materials and methods

Strain and culture conditions

Mucor rouxii ATCC 24905 strain was used in this work and was maintained as described previously [16]. Sporangiospores of *M. rouxii* were generated by growing mycelia

on polished rice grains at 30°C as described previously [18]. An amount of 10^7 spores was inoculated into 50 ml of YPG broth (pH 4.5) consisting of 0.3% (w/v) bacto-yeast extract, 1% (w/v) bacto-peptone and 2% (w/v) glucose. To induce the yeast-like form of *M. rouxii*, 0.22% (v/v) PEA was added to the medium [17, 19]. Mycelial and yeast-like cultures were grown aerobically at 30°C to logarithmic phase by shaking at 130 rpm and then shifted to 10°C. Before and after the temperature shift, aliquots were harvested by vacuum filtration (Whatman GF/C; Whatman International, Kent, UK).

Northern blot analysis

After harvesting, the cells were immediately frozen using liquid nitrogen and then ground with a cold mortar and pestle. Total RNA was extracted using TRI reagent (Molecular Research Center, Inc., Cincinnati, OH, USA). Approximately 10–15 μ g of total RNA were subjected to electrophoresis on a 1.5% (w/v) agarose gel containing 16% (v/v) formaldehyde and transferred to a nylon membrane (Hybond N, GE Healthcare, UK) as described previously [20]. The nucleotide sequences coding for Δ^9 -, Δ^{12} - and Δ^6 -desaturases of *M. rouxii* previously deposited in GenBank (Accession Nos. AY995173, AF161219 and AY392409) were used to design the DNA probes for hybridization. DNA probes were labeled with α -[P³²] dCTP using the HighPrime[®] Labeling System (Roche, Mannheim, Germany). Hybridization was performed as described previously [16]. The relative amount of each mRNA was quantified using ImageQuant TL (GE Healthcare, UK) in which 18S rRNA was used as an internal standard.

Lipid and fatty acid analyses

Lipids were extracted from mycelia and yeast cells as described previously [21]. Lipid extracts were fractionated by thin-layer chromatography (TLC), using TLC plates (20 × 20 cm) coated with silica gel 60 (Merck, Germany). NL were separated using a solvent system containing hexane/diethyl ether/acetic acid (70:30:1, by vol.) according to Certik et al. [21]. Polar lipids were fractionated with chloroform/acetone/methanol/acetic acid/H₂O (50:20:10:10:5, by vol.) [22]. Lipid classes were visualized by iodine vapor and identified by comparison of their R_f values with those of known standards (Sigma, St. Louis, MO). Individual lipid classes were relatively quantified by a densitometer using image analysis software (ImageQuant TL, GE Healthcare, UK).

Lipids were scraped off from the plates for fatty acid analysis. Fatty acid methyl esters (FAMES) were prepared as described previously [23] and then analyzed by a gas chromatography (GC-17A, Shimadzu Ltd., Tokyo, Japan)

equipped with a flame ionization detector and an OMEGAWAX™ 250 capillary column (30 m × 0.25 mm). The column temperature was programmed using the gradient mode starting at 110°C and gradually increased to 205°C at a rate of 90°C min⁻¹ and maintained at the latter temperature for 18 min. The temperature of the injector and detector were maintained at 250 and 260°C, respectively. Helium was used as a carrier gas. Individual fatty acids were identified by comparing their retention times with those of known FAME standards (Sigma, St. Louis, MO, USA). Heneicosanoic acid (C21:0) was used as an internal standard. Fatty acid amounts were quantified by calculating their chromatographic peak areas.

Data analysis

All experiments were independently carried out at least in triplicate. The statistical analysis of data was performed using program of Statistical Package for the Social Sciences (SPSS version 11.0, Chicago, IL, USA). Results were considered significant when differences had values of $P < 0.05$.

Results

Cold-induced changes of fatty acid composition in the yeast and mycelial cultures of *M. rouxii*

Differences in fatty acid composition of mycelial and PEA-induced yeast cells of *M. rouxii* grown under aerobic condition have been reported previously [17]. In this study, two major lipid classes, PL and NL of the *M. rouxii* mycelial and yeast cultures grown at 30°C were determined. Under these conditions, we detected a slight difference in compositions of PL and NL between the yeast-like and mycelial cultures. Similar to the previous study by Jackson

et al. [24] in *Mucor circinelloides*, phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were the major classes of polar lipids in the both cultures of *M. rouxii*. However, the *M. rouxii* yeast-like cells contained higher amount of PE and lower amount of PC as compared with those of the mycelial culture. NL were the main class of lipids accounting for about 83 and 87% of total lipid in yeast-like and the mycelial cells, respectively, and contained predominant fractions of steryl esters (SE), TAG and free fatty acid (FFA), whereas lower amounts of monoacylglycerol (MAG) and diacylglycerol (DAG) were detected (Table 1). A higher amount of TAG and a parallel decrease of SE were found in the *M. rouxii* mycelia compared with the yeast-like cell. Fatty acid analysis showed that C18:1 Δ^9 , which was found in high amount of the PEA-induced yeast cell (Table 2), was distributed among PL and NL classes (Tables 3, 4), whereas the lesser amount of C18:1 Δ^9 was observed in the both lipid classes of the mycelial culture. In contrast, content of saturated fatty acids (C16:0 and C18:0) in the yeast culture was significantly lower than that of the mycelial culture. Particularly, very minor amount of C18:0 (about 2% of total lipids) was found in the yeast culture. The individual fractions of PL and NL in the mycelia culture contained GLA content higher than those of the PEA-treated culture.

When the yeast and mycelial cultures grown at 30°C were shifted to lower temperature (10°C), total lipid content as represented by total fatty acids/dry weight (TFA/DW) of the PEA-induced yeast and mycelial cultures were not altered compared with those of the cultures before temperature shift (Table 2). Furthermore, there was no significant change in the proportions of individual NL and PL fractions between the cultures before and after the shift (Tables 1, 3). After the shift, the ratios of C16 and C18 fatty acids of total lipids were not significantly changed, whereas the changes were clearly observed in C18 fatty acids (Table 2). The C18:1 Δ^9 level decreased accompanied

Table 1 Neutral lipid composition in the yeast and mycelial cultures of *M. rouxii* during low temperature exposure

Culture	Composition of individual fractions in NL (%)				
	MAG	DAG	FFA	TAG	SE
Yeast					
30°C	8.8 ± 1.4	13.9 ± 2.6	23.8 ± 1.1	29.8 ± 2.1	23.7 ± 0.2
10°C 1 h	9.7 ± 0.4	13.6 ± 0.5	24.8 ± 0.3	26.3 ± 0.3	25.7 ± 0.4
10°C 2 h	10.0 ± 0.7	13.7 ± 2.5	25.4 ± 0.7	26.4 ± 2.7	24.4 ± 0.2
10°C 4 h	8.0 ± 1.5	13.9 ± 1.1	24.0 ± 3.2	27.0 ± 2.4	27.1 ± 1.0
Mycelium					
30°C	7.9 ± 0.9	14.0 ± 0.5	23.9 ± 0.4	33.4 ± 0.3	20.9 ± 0.4
10°C 1 h	6.4 ± 1.5	15.7 ± 0.6	25.4 ± 3.2	32.3 ± 3.8	20.2 ± 2.9
10°C 2 h	7.5 ± 1.9	16.8 ± 1.4	27.8 ± 5.7	29.1 ± 6.3	18.8 ± 2.0
10°C 4 h	7.3 ± 1.7	16.2 ± 0.2	25.4 ± 4.2	32.5 ± 4.5	18.6 ± 2.6

Table 2 Fatty acid composition of total lipid in the yeast and mycelial cultures of *M. rouxii* during low temperature exposure

Culture	Fatty acid composition (%)								TFA/DW (% w/w)
	C16:0	C16:1 Δ^9	C18:0	C18:1 Δ^9	C18:2 $\Delta^{6,9}$	C18:2 $\Delta^{9,12}$	GLA	Others	
Yeast									
30°C	8.2 ± 0.2	3.1 ± 0.3	1.7 ± 0.1	49.1 ± 0.6	11.1 ± 0.6	11.3 ± 0.8	13.8 ± 0.5	1.6 ± 0.0	7.3 ± 0.2
10°C 1 h	8.5 ± 0.0	3.2 ± 0.1	1.7 ± 0.3	46.6 ± 2.6	10.1 ± 1.4	13.1 ± 2.0	15.1 ± 2.5	1.7 ± 0.0	6.5 ± 0.1
10°C 2 h	8.6 ± 0.7	3.1 ± 0.4	1.3 ± 0.2	43.1 ± 3.0	11.4 ± 2.3	12.9 ± 0.7	17.4 ± 0.5	2.1 ± 0.2	6.9 ± 0.7
10°C 4 h	9.4 ± 0.1	4.5 ± 1.3	2.1 ± 0.1	43.5 ± 0.3	8.6 ± 1.3	14.5 ± 1.2	15.4 ± 2.2	2.4 ± 0.5	7.6 ± 1.1
Mycelium									
30°C	18.7 ± 0.2	4.6 ± 0.5	12.2 ± 0.8	27.4 ± 0.5	3.0 ± 0.0	11.1 ± 0.3	16.8 ± 1.1	6.2 ± 0.7	6.7 ± 0.3
10°C 1 h	18.2 ± 0.5	5.2 ± 0.4	11.0 ± 1.1	26.5 ± 1.3	3.4 ± 0.6	11.0 ± 0.6	17.9 ± 0.9	6.7 ± 0.7	6.4 ± 0.7
10°C 2 h	18.4 ± 0.1	5.4 ± 0.3	10.8 ± 1.0	25.4 ± 0.2	2.8 ± 0.1	12.1 ± 0.3	19.0 ± 0.3	6.1 ± 0.2	6.9 ± 0.0
10°C 4 h	17.9 ± 0.5	5.8 ± 0.3	8.5 ± 0.9	25.0 ± 2.1	3.6 ± 0.0	11.0 ± 1.3	20.9 ± 0.0	7.2 ± 0.9	5.8 ± 0.3

Table 3 Content of PC and PE in total phospholipid, and fatty acid composition of the individual fractions in the yeast and mycelial cultures of *M. rouxii* during low temperature exposure

Polar lipid	Culture	Content in total PL (%)	Fatty acid composition (%)							
			C16:0	C16:1 Δ^9	C18:0	C18:1 Δ^9	C18:2 $\Delta^{6,9}$	C18:2 $\Delta^{9,12}$	GLA	Others
Yeast										
PE	30°C	24.6 ± 2.6	11.6 ± 2.0	4.2 ± 0.7	0.6 ± 0.1	43.5 ± 1.8	19.1 ± 1.5	6.9 ± 1.6	11.0 ± 1.1	3.1 ± 0.9
	10°C 1 h	25.4 ± 3.9	11.2 ± 1.6	4.7 ± 0.3	0.5 ± 0.1	42.6 ± 0.9	18.9 ± 2.8	8.3 ± 1.2	10.7 ± 1.1	3.1 ± 0.6
	10°C 2 h	27.4 ± 3.4	10.3 ± 1.4	3.8 ± 0.5	0.5 ± 0.2	40.6 ± 0.8	16.8 ± 1.4	10.0 ± 0.9	15.5 ± 1.6	2.6 ± 0.3
	10°C 4 h	27.8 ± 2.6	13.4 ± 4.1	5.1 ± 0.2	1.0 ± 0.6	35.3 ± 3.8	14.5 ± 3.8	12.2 ± 0.5	14.7 ± 0.1	3.9 ± 2.2
PC	30°C	20.0 ± 3.9	4.8 ± 0.7	2.5 ± 0.1	1.3 ± 0.2	55.8 ± 0.9	17.4 ± 1.8	8.4 ± 0.9	8.2 ± 0.7	1.7 ± 0.6
	10°C 1 h	22.6 ± 3.9	5.9 ± 0.1	2.4 ± 0.1	1.4 ± 0.2	47.5 ± 0.7	15.0 ± 0.2	14.5 ± 0.0	11.2 ± 0.3	2.1 ± 0.4
	10°C 2 h	22.2 ± 3.9	6.0 ± 0.9	2.9 ± 0.3	1.0 ± 0.4	43.3 ± 1.2	16.5 ± 0.2	12.4 ± 0.6	16.1 ± 2.0	1.7 ± 0.2
	10°C 4 h	21.4 ± 3.0	4.5 ± 1.1	3.5 ± 0.5	1.0 ± 0.6	41.8 ± 1.4	16.2 ± 1.9	16.6 ± 0.7	14.9 ± 0.3	1.7 ± 0.2
Mycelium										
PE	30°C	20.0 ± 3.3	15.2 ± 0.7	13.4 ± 0.4	1.6 ± 0.2	15.8 ± 0.8	4.1 ± 0.4	17.0 ± 1.9	19.7 ± 0.7	13.1 ± 2.0
	10°C 1 h	20.7 ± 3.2	15.0 ± 0.8	12.7 ± 1.3	1.5 ± 0.1	15.7 ± 3.5	4.6 ± 0.2	17.4 ± 0.0	21.3 ± 2.3	11.7 ± 3.8
	10°C 2 h	21.0 ± 1.7	16.4 ± 3.7	11.5 ± 1.8	1.8 ± 0.5	16.6 ± 2.7	4.1 ± 1.1	18.0 ± 2.4	22.2 ± 0.7	9.3 ± 0.2
	10°C 4 h	20.7 ± 2.8	14.9 ± 1.5	11.8 ± 1.6	1.6 ± 0.4	14.0 ± 1.3	4.2 ± 1.3	18.1 ± 1.9	25.6 ± 1.6	9.8 ± 1.6
PC	30°C	24.1 ± 2.5	17.3 ± 1.5	11.1 ± 0.2	5.3 ± 0.4	18.6 ± 1.5	3.3 ± 0.6	21.4 ± 0.6	17.3 ± 0.8	5.8 ± 0.5
	10°C 1 h	25.4 ± 2.0	16.1 ± 4.5	10.5 ± 1.8	4.9 ± 1.4	15.9 ± 3.0	3.6 ± 0.1	22.1 ± 3.3	21.5 ± 3.8	4.9 ± 0.0
	10°C 2 h	25.2 ± 1.9	15.9 ± 8.0	10.4 ± 3.4	4.6 ± 2.0	14.9 ± 2.5	3.3 ± 1.1	21.2 ± 3.2	24.8 ± 4.9	3.7 ± 1.8
	10°C 4 h	25.6 ± 2.2	16.0 ± 3.5	10.7 ± 1.3	4.0 ± 1.0	12.3 ± 0.8	2.2 ± 0.3	23.1 ± 1.6	26.8 ± 3.7	4.8 ± 0.9

by a proportional increase of PUFAs (C18:2 $\Delta^{9,12}$ and GLA) after exposure to low temperature for about 2 h. These results were similar in both yeast and mycelial cultures. As expected, the content of saturated fatty acids of the mycelial culture decreased after temperature downshift. The changes of fatty acid composition in the both PEA-induced yeast and mycelial cultures induced by lowering the temperature were also clearly observed in particular lipid classes, PE and PC, which were the major constituents of PL class (Table 3). In addition to PL, cells responded to the low temperature by increasing PUFA proportion in

MAG and DAG, which comprised the minor part of NL (Table 4). It is worth mentioning that fatty acid composition of typically storage lipids, TAG and steryl esters did not change significantly (data not shown).

Transcriptional response to temperature downshift of desaturase genes in mycelial and yeast-like cells of *M. rouxii*

We examined the expression of Δ^9 -, Δ^{12} - and Δ^6 -desaturases in PEA-induced yeast and mycelial cells of *M. rouxii*,

Table 4 Fatty acid composition of MAG and DAG fractions in the yeast and mycelial cultures of *M. rouxii* during low temperature exposure

Neutral lipid	Culture	Fatty acid composition (%)							
		C16:0	C16:1 Δ^9	C18:0	C18:1 Δ^9	C18:2 $\Delta^{6,9}$	C18:2 $\Delta^{9,12}$	GLA	Others
Yeast									
MAG	30°C	16.8 ± 3.3	2.8 ± 0.3	0.8 ± 0.0	47.7 ± 0.0	9.8 ± 0.0	8.4 ± 2.1	10.3 ± 0.9	3.5 ± 0.0
	10°C 1 h	11.3 ± 0.8	3.0 ± 0.4	0.6 ± 0.2	43.9 ± 0.8	15.0 ± 1.4	10.6 ± 0.4	12.7 ± 1.0	2.8 ± 0.2
	10°C 2 h	11.9 ± 0.4	2.6 ± 0.3	0.5 ± 0.0	38.3 ± 1.2	12.9 ± 2.9	13.3 ± 1.0	17.3 ± 0.3	3.2 ± 1.2
	10°C 4 h	13.0 ± 0.0	3.6 ± 0.2	0.8 ± 0.3	36.8 ± 2.9	11.8 ± 1.2	15.1 ± 0.2	15.8 ± 1.4	3.0 ± 0.9
DAG	30°C	4.6 ± 0.0	2.1 ± 0.1	2.6 ± 0.8	56.6 ± 0.0	15.7 ± 0.0	4.8 ± 0.8	9.2 ± 0.5	4.4 ± 0.9
	10°C 1 h	4.6 ± 1.3	2.2 ± 0.1	2.2 ± 0.8	50.7 ± 0.6	14.7 ± 4.1	7.0 ± 0.2	13.8 ± 1.0	4.8 ± 2.5
	10°C 2 h	7.5 ± 0.0	3.8 ± 0.6	2.9 ± 0.5	47.5 ± 0.0	11.0 ± 1.6	6.0 ± 1.9	13.1 ± 0.0	8.2 ± 1.6
	10°C 4 h	5.0 ± 0.6	3.3 ± 0.6	2.4 ± 0.0	53.1 ± 0.1	10.6 ± 1.7	6.9 ± 2.2	14.4 ± 0.0	4.3 ± 0.7
Mycelium									
MAG	30°C	20.8 ± 0.7	5.5 ± 0.4	8.8 ± 0.3	14.0 ± 1.0	4.6 ± 0.4	14.4 ± 0.9	21.0 ± 0.0	11.0 ± 3.0
	10°C 1 h	18.9 ± 1.0	6.3 ± 0.7	9.2 ± 3.1	15.2 ± 0.8	4.5 ± 0.5	15.2 ± 1.1	21.0 ± 1.7	9.7 ± 1.4
	10°C 2 h	18.4 ± 0.4	5.4 ± 0.4	6.7 ± 0.2	15.3 ± 0.1	4.2 ± 0.2	15.4 ± 0.6	22.5 ± 0.1	12.0 ± 0.0
	10°C 4 h	17.2 ± 0.2	5.2 ± 0.5	7.6 ± 1.7	11.8 ± 1.3	4.8 ± 0.5	14.2 ± 0.2	26.2 ± 1.0	13.0 ± 0.4
DAG	30°C	11.6 ± 0.0	7.9 ± 1.7	9.2 ± 0.6	13.2 ± 2.0	6.1 ± 1.6	12.6 ± 0.0	24.9 ± 1.6	14.5 ± 4.2
	10°C 1 h	11.7 ± 0.2	6.9 ± 0.1	8.3 ± 0.4	13.6 ± 0.5	6.5 ± 0.2	13.8 ± 0.3	28.2 ± 0.7	11.0 ± 0.2
	10°C 2 h	14.0 ± 2.7	7.5 ± 1.7	9.9 ± 0.9	11.7 ± 0.5	6.5 ± 1.1	14.4 ± 2.5	25.3 ± 0.0	10.7 ± 7.7
	10°C 4 h	11.6 ± 0.6	6.1 ± 0.6	8.1 ± 0.2	10.5 ± 1.2	6.1 ± 0.7	14.5 ± 0.8	31.3 ± 1.5	11.8 ± 3.9

that contained different fatty acid compositions, in response to temperature stress. Total RNA was extracted from cells before (30°C) and after the temperature downshift (10°C) at different time points. Figure 1 shows by Northern blot analysis a rapid and transient increase of transcript levels of the three desaturases in the yeast culture after the temperature shift. The increased mRNA levels could be observed after a 30 min temperature shift and reached maximum amount 1 h after the temperature change. Then, transcript levels gradually decreased to the levels present before the shift. Although lowering temperature induced the expression of the three desaturase genes in the yeast culture of *M. rouxii*, the level of Δ^9 -desaturase transcript increased highly when compared with that of the Δ^6 - and Δ^{12} -desaturases, which are involved in the synthesis of PUFAs.

The expression of the three desaturase genes was rapidly induced in mycelia after the shift to the low temperature. The transient increases of desaturase mRNA levels were also found (Fig. 2) to be similar to the transcriptional responses of the yeast-like culture. However, there were subtle changes of expression of desaturase genes between mycelial and yeast cultures during cold acclimation. While Δ^9 -desaturase transcript of the yeast-like culture was substantially induced by lower temperature, the up-regulated expression of Δ^{12} -desaturase gene was the main class found in mycelia under the same temperature treatment. In mycelia, a 4-fold increase of Δ^{12} -desaturase mRNA level

was observed while a 2–2.5-fold expression was measured for Δ^9 - and Δ^6 -desaturase transcripts.

Discussion

Fatty acids in general are mostly esterified to specific moieties and incorporated into different compartments of cells. Fatty acids not only have structural roles in cellular membrane, but are also dynamic molecules that have a important roles when cells are exposed to environmental stresses and during homeoviscous adaptation. This study focused on the cold-induced response underlying fatty acid metabolism in the dimorphic fungus, *M. rouxii*. We found that mycelial and PEA-induced yeast cultures of *M. rouxii* having different fatty acid profiles acclimated similarly to low temperature by enhancing fatty acid desaturation through cooperative up-regulation of expression of desaturase genes yielding increased levels of C18:2 $\Delta^{9,12}$ and GLA. The relative increase of unsaturated fatty acids, particularly in major classes of PL (PE and PC) in the *M. rouxii* cultures implies that membrane lipids might be modulated to maintain physical properties of cellular membrane during adaptation to the temperature change. The increase of membrane fluidity under such experimental condition is a widespread phenomenon that has been found in both prokaryotic and eukaryotic organisms [25–29]. In temperature-shifted cultures, fatty acid composition of

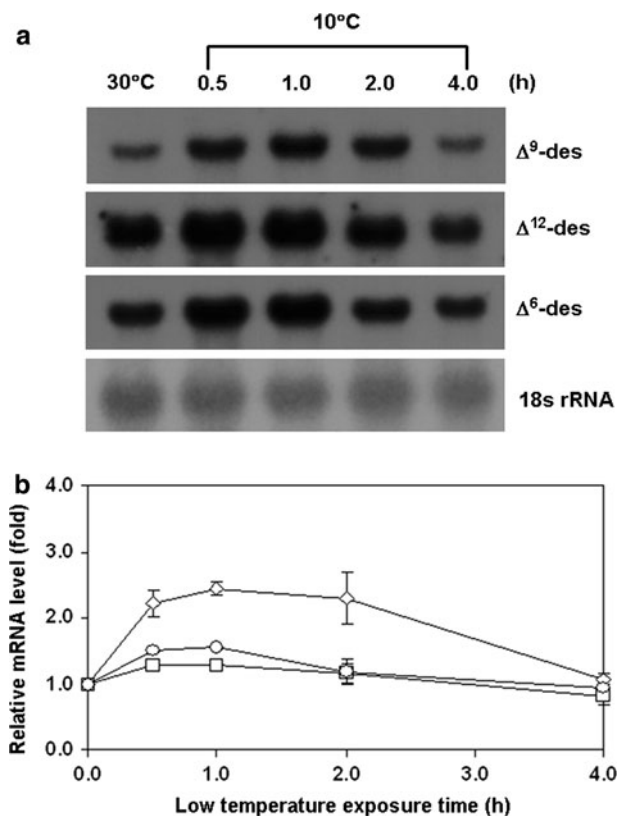


Fig. 1 Expression of Δ^9 -, Δ^{12} - and Δ^6 -desaturase genes of the *M. rouxii* yeast-like cultures in response to low temperature. Cells were harvested before (30°C) and after temperature downshift (10°C) at different time points and then used for total RNA extraction. Autoradiograph signals of the desaturase transcripts (a) were quantified using 18S as an internal standard to derive the relative mRNA levels (b) of the Δ^9 -desaturase (*diamond*), Δ^{12} -desaturase (*square*) and Δ^6 -desaturase genes (*circle*)

DAG and MAG, which are minor constituents in NL, was altered in manner similar to that of PE and PC. This finding may be explained by the fact that DAG is an intermediate in lipid metabolism and is present in all membranes [30]. DAG is substrate for the synthesis of PE and PC and it can be produced starting from MAG or phosphatidic acid. Although DAG is also a direct precursor for TAG synthesis, fatty acid composition of TAG in the shifted cultures did not significantly change as compared to that of the cultures before the shift. One possible explanation is that TAG may not be a primary target in response to temperature change of *M. rouxii* due to its widespread presence in lipid bodies as energy storage.

Genetic variations of fatty acid metabolism induce considerable diversity of fatty acid composition in each organism. Not surprisingly, it has been reported that the temperature-induced alteration of fatty acid composition greatly differs among fungal species [31] despite the existence of common response of membrane lipid to temperature adaptation. We also found in *M. rouxii* subtle

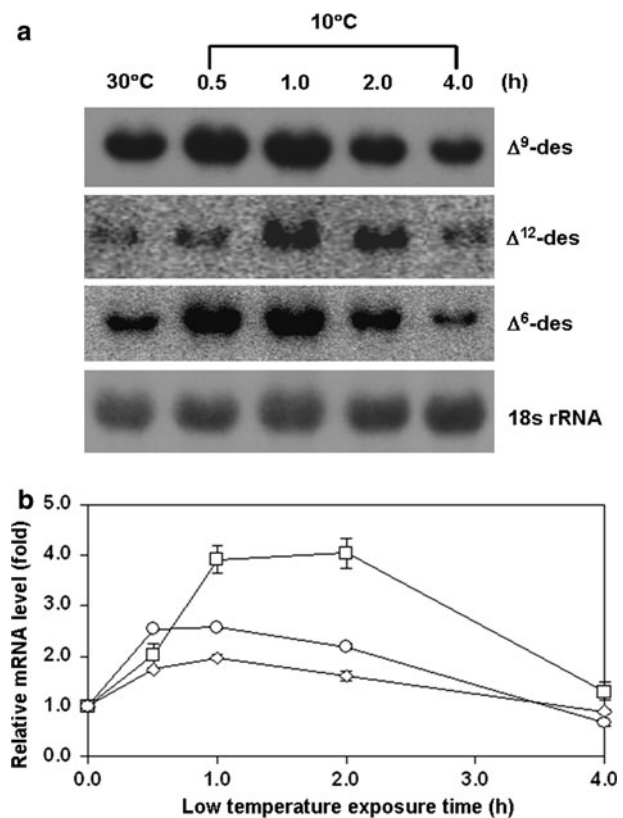


Fig. 2 Expression of Δ^9 -, Δ^{12} - and Δ^6 -desaturase genes of the *M. rouxii* mycelial cultures in response to low temperature. Cells were harvested before (30°C) and after temperature downshift (10°C) at different time points and then used for total RNA extraction. Autoradiograph signals of the desaturase transcripts (a) were quantified using 18S as an internal standard to derive the relative mRNA levels (b) of the Δ^9 -desaturase (*diamond*), Δ^{12} -desaturase (*square*) and Δ^6 -desaturase genes (*circle*)

difference in the thermal response of fatty acid desaturation at transcription level in yeast and mycelial cells, although the response to low temperature via the modulation of fatty acid composition appeared similar in those cultures as mentioned earlier. After cold shock, a lower increase of transcript levels of desaturases (about 1.2- to 2.5-fold increases) was detected in yeast cultures as compared with the up-regulation of desaturase transcripts (about 2- to 4-fold increases) in the mycelial culture. It seems likely that the oleate-rich yeast cell could tolerate low temperature by fine adjustment of the transcriptional regulation of desaturation. This might be due to the pre-existing fatty acid composition and different membrane fluid state [2] of *M. rouxii* yeast cells that accumulate substantial amounts of unsaturated fatty acids (about 88% of total fatty acid) in which C18:1 Δ^9 was the predominant molecular species. It has been reported that the introduction of the first double bond at Δ^9 -position in acyl chain has the greatest effect on membrane physical properties [32, 33]. We can not exclude, however, that other factors present in mycelia and

yeast may regulate differently the expression of the different desaturases. Probably, each desaturase gene may be regulated differently in mycelia and yeast of *M. rouxii* by, for example, DNA binding proteins differently regulated and other co-factors. However, the transient response of expression of desaturase genes to acclimate efficiently to low temperature that we found in both cultures of *M. rouxii* is in agreement with previous reports [34–36].

In conclusion, *M. rouxii* cells displayed a responsive mechanism to temperature downshift by increasing desaturation in membrane lipids through transcriptional expression of Δ^9 -, Δ^{12} - and Δ^6 -desaturase genes. The pre-existing fatty acid constituents of the yeast-like and mycelial cells may influence a subtle difference in the transcriptional response of the three desaturase genes.

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