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Lipidomic analysis of psychrophilic yeasts cultivated at different temperatures

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ABSTRACT

Analysis of polar lipids from eight psychrophilic yeasts (*Cryptococcus victoriae*, *Cystofilobasidium capitatum*, *Holtermaniella wattica*, *Mrakiella aquatica*, *M. cryoconiti*, *Rhodotorula lignophila*, *Kondoa malvinella* and *Trichosporon aggtelekiense*) grown at 4–28 °C by hydrophilic interaction liquid chromatography/high resolution electrospray ionization tandem mass spectrometry determined 17 classes of lipids and identified dozens of molecular species of phospholipids including their regioisomers. Most of the yeasts were able to grow over the whole temperature range, reaching the highest biomass at 4 or 10 °C. On temperature drop to 4 °C, all eight strains showed a significant decrease of MUFA and a simultaneous increase of PUFA such as α-linolenic acid, the content of which in the biomass reached up to 20%. We also found alterations in the proportions of individual phospholipids (PI, PE and PC), the PC/PE-ratio decreasing with decreasing temperature. With increasing temperature the content of PoO-PC rose while that of LL-PC decreased, the drop in the content of LL-PC being nearly 100-fold while the content of PoO-PC increased more than twice.

A change in temperature brought about changes in molecular species of PC (molecular species PO-PC versus OP-PC) as well as PE, i.e. PO-PE and OP-PE. The phase transition temperature of PO-PC differs from OP-PC by 7 °C and the difference between PO-PE and OP-PE is some 10 °C; we thus assume that the cell compensates for the adverse temperature effect by changing the fatty acids in the *sn*-1 and *sn*-2 positions.

1. Introduction

The ability of microorganisms to adapt to different temperatures at which they grow is fascinating. Some microorganisms were found to be capable of growing at extreme temperatures [1]. Many studies in this field that concern yeast [2] deal with ecology and more particularly taxonomy [3] while only a few describe changes of lipids and fatty acids, especially their unsaturation in psychrophilic yeast. Yeast can grow in a broad range of temperatures. This is nicely documented in one of the most common industrial processes using yeasts - production of beer - wherein optimum fermentation temperature for top brewer's yeast can reach more than 20 °C whereas for the closely related bottom brewer's yeast it is around 10 °C [4]. Essential structural components of yeast membranes are phospholipids. The main phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA). The major fatty acids (FA) found in these phospholipids are palmitic (P; 16:0), palmitoleic (Po; 9–16:1), stearic (S; 18:0) and oleic (O; 9–18:1). Minor acids include medium length FA such as caprylic (Cy; 8:0), capric (C; 10:0), lauric (L; 12:0) and myristic (M; 14:0) acid, unsaturated acids such as gadoleic (G; 20:1), linoleic (L; 9,12–18:2) and α-linolenic acid (Ln; 9,12,15–18:3) and/or very-long chain FA, i.e. behenic (B; 22:0), lignoceric (Lg; 24:0), cerotic (Ce; 26:0) acid and many others. Among other constituents are odd-numbered FA - pentadecanoic (15:0) or pentadecenoic (15:1) acids [5,6]. Other components of yeast membrane lipids are neutral lipids, particularly sterols, especially ergosterol and its esters with FA [7].

The separation of complex lipid mixtures such as yeast lipids makes use of a number of methods [8,9]. One of the most effective ones is based on the direct entry of the sample into the mass spectrometer, often a high resolution mass spectrometer. Besides the advantages such as speed, accuracy, or low demand for processing and sample preparation the method has some drawbacks. The major advantage of liquid chromatography-mass spectrometry (LC/MS) analysis over shotgun lipidomics is the usage of chromatographic separation to simplify the complex lipid extracts. We therefore used the method of LC/MS, more specifically hydrophilic interaction liquid chromatography (HILIC), which is a variant of normal phase liquid chromatography. Due to the nature of the analyzed lipids, we preferred high resolution electrospray (ESI) mass spectrometry in positive or negative mode, which is commonly used in the analysis of lipids [10,11]. HILIC separates lipids on the basis of their polar head groups whereas separation according to chain length, number and configuration of double bonds, etc., is only

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partial. We used an HILIC column for separating different lipid classes and high resolution mass spectrometry (HR-MS), positive and/or negative ESI for identification of molecular species including regioisomers of appropriate lipid classes in 8 psychrophilic yeasts cultured at various temperatures.

2. Material and methods

2.1. Chemicals and standards

Acetonitrile, 2-propanol, hexane, dichloromethane, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (OP-PE) and ergosterol (Es) were purchased from Sigma-Aldrich (Prague, CR). Cholesteryl oleate (SE), triolein (TG), diolein (DG), oleic acid (FA), 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (PC), 1,2-dioleoyl-*sn*-phosphatidylethanolamine (PE), 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt) (PS), 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine (LPC), 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphatidic acid, Na-salt (LPA), and 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl-rac-glycerol (Na-salt) (PG), 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt) (PS) were from Larodan (Malmö, Sweden). 1,2-Dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol) (ammonium salt) (PI) and 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′-phosphate) (ammonium salt) (PIP), 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-myo-inositol-3′,4′,5′-trisphosphate) (ammonium salt) (PIPs), 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (18:1-16:0-PC, respective OP-PC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16:0-18:1-PC, respective PO-PC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (PO-PE) were purchased from Avanti (Avanti Polar Lipids, Inc., Alabama, USA).

2.1.1. Cultivation-microorganisms

The yeast strains used in the present study were *Cryptococcus victoriae* (DBVPG 4826), *Cystofilobasidium capitatum* (DBVPG 4845), *Holtermaniella wattica* (DBVPG 5411), *Mrakiella aquatica* (DBVPG 4979), *Mrakiella cryoconiti* (DBVPG 5179), *Rhodotorula lignophila* (DBVPG 7029) and *Kondoa malvinella* (DBVPG 7023). All were supplied by DBVPG - Industrial yeast collection, Department of Agricultural, Food and Environmental Science, University of Perugia (Italy). For long term storage the stock cultures were maintained in 20% glycerol at −70 °C. Malt extract agar (23 g/L, pH 7) was employed for short term storage. *Trichosporon aggtelekiensis* (MycoBank MB814052) was donated by Dr. A. Novakova from the Institute of Microbiology [12].

2.1.2. Cultivation conditions

The pre-cultures of yeast strains were cultivated in 200 mL of YPD medium (20 g/L peptone, 10 g/L yeast extract, 20 g/L glucose, initial pH 6.0, sterilized at 121 °C for 20 min) in Erlenmeyer flasks at 10 °C on a rotary shaker at 150 rpm to the late exponential growth phase. For lipid production, 200 mL volume of YPD medium in 500 mL Erlenmeyer flasks was inoculated with 10 mL of pre-culture to a final concentration of OD_{600} 0.2 and incubated on a rotary shaker at 150 rpm and different temperatures (4 °C; 10 °C; 20 °C and 28 °C) in triplicate parallels.

The cultivations were carried out from 3 to 8 days (until early stationary phase) depending on cultivation temperature. After cultivation, the cells were centrifuged (9000 g, 10 min) and washed two times. Biomass yield was determined as dry cell weight. Biomass was frozen at −75 °C and lyophilized [13].

2.2. Isolation of lipids

Isolation and pretreatment of lipids was carried out according to Bligh and Dyer [14], with modifications as described previously [15]. For full description of the experiments see the Supplements.

2.3. FAMEs analysis

Analysis of fatty acids in the form of methyl esters (FAME) was described previously [16,17]. A complete description of all conditions of FAME analysis by GC/MS is in the Supplements.

2.4. HILIC-LC/MS-ESI

The total lipids were identified and quantified by LC/MS. A detailed description of negative ESI is again described in the Supplements.

2.5. Statistics

To perform multivariate statistical analyses, we used the program CANOCO 5 (Microcomputer Power, USA). Since the gradient lengths in the data were short, we used a principal component analysis (PCA) to visualize the variability within the dataset.

Three replicates ($n = 3$) of each cultivation, at 4 °C; 10 °C; 20 °C and 28 °C temperatures, were used. The software Statistica for Windows (version 12.0, Dell) was used for analyses. Data were presented as means ± SD.

3. Results

3.1. Identification and production of fatty acids

Table 1 gives the contents of fatty acids (FA) in polar lipids in the 8 yeast strains cultivated at different temperatures (4 °C; 10 °C; 20 °C and 28 °C). Only major FA are given and the data are given as means ± S.D. The more extensive Table 1S (Supplements) contains data on all identified FA; for space constraints the data are given without ± S.D., which in minor FA reach a maximum of ± 20 relative percent. The content of individual acids, as dependent on the temperature of cultivation, was not much different. Analysis and identification of FA, see Table 1, were done routinely. Apart from common major acids such as palmitic, palmitoleic, stearic, oleic, linoleic and α-linolenic acids, the analysis documented the presence of odd-numbered FA, e.g. margaric or margaroleic acids, minor short-chain FA with less than C16 (myristic, myristoleic) and, on the other hand, FA with more than C18 (arachidic, gadoleic, behenic, erucic, and lignoceric acids), which are commonly found in some yeasts. Table 1 and Fig. 1 show that the content of monounsaturated FA, mainly oleic acid, strongly varies in dependence on cultivation temperature and yeast species.

As seen in Fig. 1, the content of monounsaturated FA, predominantly oleic acid, decreases with increasing temperature in several yeasts, i.e. in the genus *Mrakiella*, remains virtually unchanged in *K. malvinella* and drops in *R. lignophila*. In order to verify these trends, we performed the Principal Component Analysis (PCA) of fatty acid composition in all yeast species cultivated at four different temperatures. Fig. 2 shows that of the five major acids (P, S, O, L and Ln) four are in line with the commonly accepted hypothesis about the influence of unsaturation of FA on the fluidity of the membranes under changing temperatures, the only exception being oleic acid where the first two canonical axes are plotted; the first axis explained 54.2% and the second 28.0% of the variability. We therefore focused on lipids containing these acids.

3.2. Separation and identification of polar lipids

We used two approaches, analysis of polar lipids using LC/MS and also analysis of molecular species of phospholipids, including lysophospholipids and their regioisomers (see below). The commonly used methods of analysis of lipids include TLC and HPLC and the connection of HPLC with mass spectrometer is preferred.

The chromatograms in Fig. 1S demonstrate a base-line separation of all major lipid classes (PC, PE, PG, PI, PS, and PA, including

Table 1
Fatty acid profile (%w/w) of eight yeast strains cultivated at different temperatures. For the content of all identified FA see Table 1S.

Strain	Temp.	P	±S.D.	S	±S.D.	O	±S.D.	L	±S.D.	Ln	±S.D.	Minority FA
C. victoriae	4 °C	17.8	1.3	4.1	0.7	33.2	1.4	19.7	1.0	19.2	1.3	6.0
	10 °C	30.1	1.5	6.2	0.5	29.9	1.2	15.2	0.9	13.6	1.0	5.0
	20 °C	32.6	1.2	9.7	1.0	36.0	1.3	11.2	0.6	7.1	0.9	3.4
	28 °C	34.9	2.1	13.6	0.9	36.4	1.0	7.6	0.7	3.8	0.4	3.7
C. capitatum	4 °C	13.7	0.8	1.7	0.2	35.6	0.9	28.5	0.9	15.2	1.0	5.3
	10 °C	21.1	0.9	6.3	0.4	32.7	1.5	25.6	1.3	10.0	0.8	4.3
	20 °C	32.0	1.2	12.6	0.8	29.5	1.1	16.7	1.0	5.9	0.4	3.3
	28 °C	39.0	1.3	23.1	1.3	22.0	1.0	10.4	1.2	1.7	0.3	3.8
H. wattica	4 °C	16.8	1.0	0.7	0.3	41.3	0.9	23.0	1.4	12.9	0.9	5.3
	10 °C	21.1	0.8	2.9	0.4	40.2	0.8	23.8	1.3	7.6	0.6	4.4
	20 °C	22.2	0.7	9.6	0.7	39.9	1.4	23.8	1.4	2.1	0.4	2.4
	28 °C	22.6	1.0	18.9	1.1	35.5	1.3	19.5	1.0	0.7	0.3	2.8
K. malvinella	4 °C	24.3	1.5	0.4	0.2	24.4	0.8	33.5	1.5	12.7	1.0	4.7
	10 °C	25.5	1.2	6.5	0.4	27.2	0.9	24.8	1.2	11.8	0.9	4.2
	20 °C	36.4	1.3	9.7	0.7	28.7	1.2	14.3	0.9	4.9	0.5	6.0
	28 °C	42.0	1.6	12.5	0.9	27.8	1.1	6.5	0.8	3.0	0.4	8.2
M. aquatica	4 °C	14.1	1.0	1.8	0.3	23.8	0.8	42.1	1.4	14.4	1.4	3.8
	10 °C	19.1	0.9	2.2	0.2	34.6	1.3	31.7	1.2	9.2	0.8	3.2
	20 °C	22.2	0.8	6.6	0.5	48.4	1.0	15.8	1.0	4.7	0.5	2.3
	28 °C	27.3	1.3	10.3	0.6	52.7	1.4	5.0	0.8	1.7	0.2	3.0
M. cryoconiti	4 °C	11.5	0.7	1.8	0.3	41.7	1.3	33.4	1.3	5.4	0.3	6.2
	10 °C	22.2	0.9	5.2	0.5	54.3	1.5	11.4	1.0	1.8	0.2	5.1
	20 °C	22.3	0.7	7.6	1.0	63.9	1.2	1.2	0.4	1.1	0.4	3.9
	28 °C	21.1	1.1	8.0	1.0	66.0	1.5	0.8	0.3	0.7	0.2	3.4
R. lignophila	4 °C	10.1	0.9	5.5	0.8	38.5	1.3	32.3	1.3	7.9	1.0	5.7
	10 °C	19.5	1.3	8.4	1.1	30.8	0.9	30.7	1.2	5.2	0.9	5.4
	20 °C	26.7	1.2	10.2	0.4	27.9	1.0	26.4	1.0	3.6	0.3	5.2
	28 °C	37.4	0.9	15.9	1.3	21.2	1.0	18.1	0.9	1.4	0.2	6.0
T. aggtelekiense	4 °C	15.2	0.8	1.6	0.4	35.4	1.3	25.9	1.3	10.4	0.6	11.5
	10 °C	23.7	0.7	4.1	0.4	33.4	1.0	21.4	1.2	7.7	0.4	9.7
	20 °C	45.0	1.4	4.4	0.6	33.4	1.2	9.2	0.9	1.1	0.2	6.9
	28 °C	47.6	1.5	6.4	0.8	38.6	1.4	2.6	0.4	0.7	0.2	4.1

Abbreviations: P-palmitic; Po-palmitoleic; S-stearic; O-oleic; L-linoleic; Ln-linolenic acids.

lysophosphatidylcholine and lysophosphatidic acid – LPC and LPA, respectively). This did not hold for triacylglycerols and diacylglycerols; we did not optimize the separation of the latter because we were interested only in cell membrane phospholipids. Identification was performed using total ion current in negative mode; individual lipid classes were identified based on precursor and neutral loss

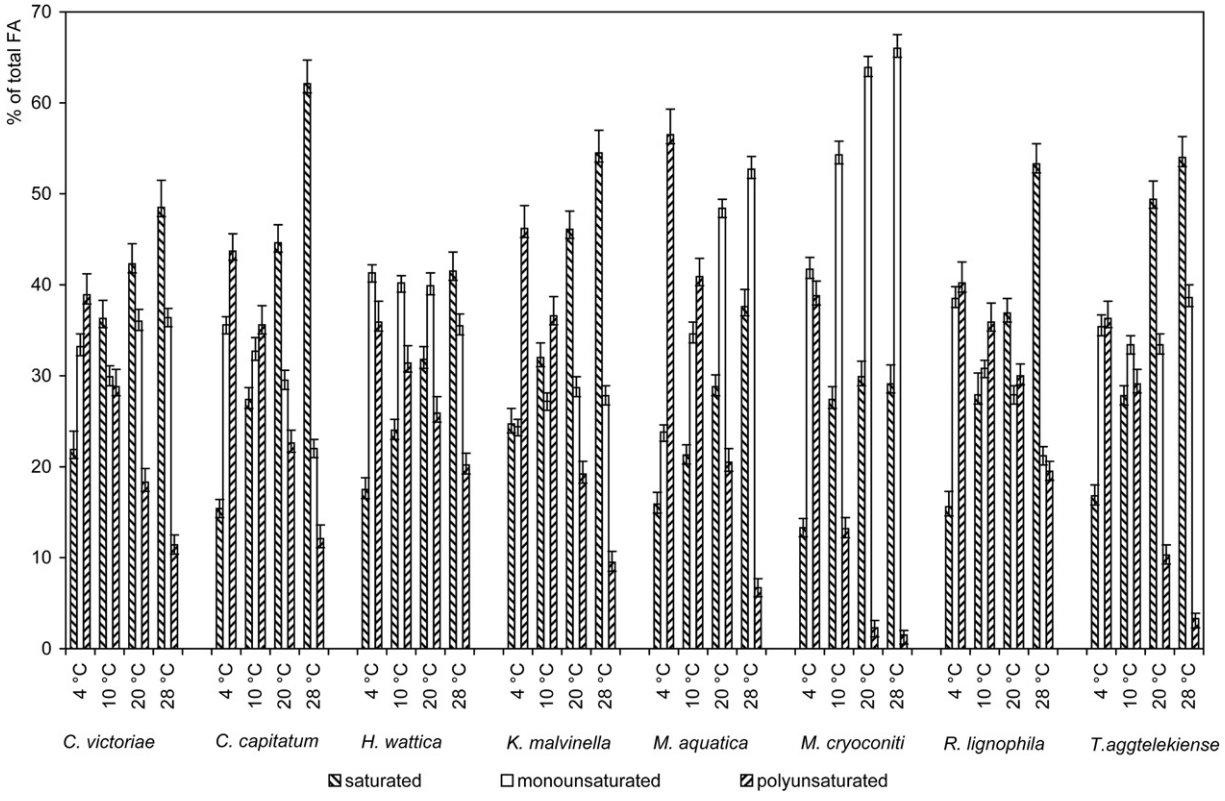

Fig. 1. Dependence of the proportion of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids on cultivation temperature in the eight yeast strains.

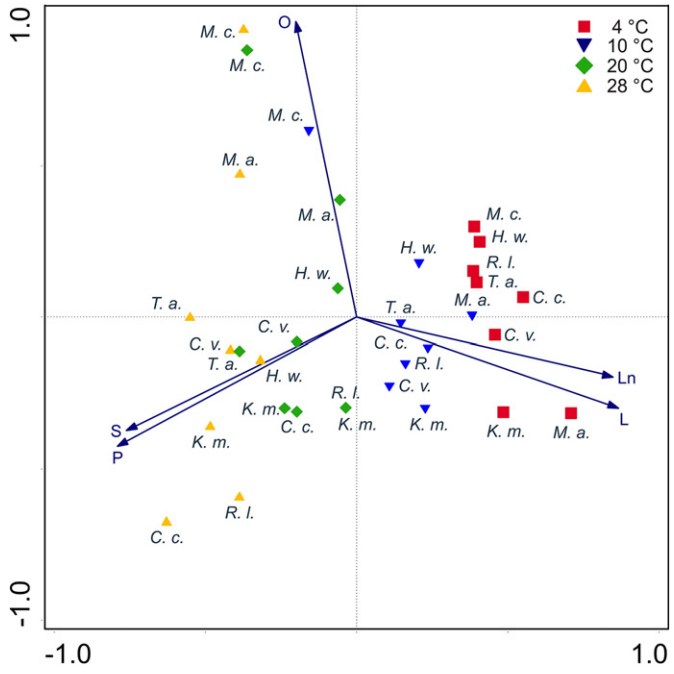

Fig. 2. Principal component analysis (PCA) of fatty acid composition in the eight yeast species cultivated at four different temperatures. The first two canonical axes are plotted; the first axis explained 54.2% and the second 28.0% of the variability. Microorganisms: *C. victoriae* (*C. v.*), *C. capitatum* (*C. c.*), *H. wattica* (*H. w.*), *K. malvinella* (*K. m.*), *M. aquatica* (*M. a.*), *M. cryoconiti* (*M. c.*), *R. lignophila* (*R. l.*), and *T. aggtelekiense* (*T. a.*). Fatty acids: palmitic (P), stearic (S), oleic (O), 1inoleic (L), linolenic (Ln).

scanning utilizing the characteristic fragments generated by negative ESI.

Separation of both commercially obtained lipid standards and lipids of *K. malvinella*, see Material and methods, was performed on a diol column by gradient elution. Base-line separation of all classes of lipids (Fig. 1S) was carried out for 60 min in negative mode (including PC) and the lipids were checked as $[M+CH_3COO]^-$ and $[M-H]^-$ ions. TAG and DAG were poorly retained on HILIC column and were eluted at the start of the chromatogram (tR = 11.3 min). As evident from Fig. 1S, diol phase provides partial intra-class separation leading to individual molecular species. This effect is particularly noticeable in those molecular species that differ significantly either in chain length or in the number of double bonds. However, unlike inter-class separation, intra-class separation into individual molecular species is insufficient.

Even so, separation on HILIC column was sufficient to separate the different classes of phospholipids. Fig. 3a–h shows that the content of major phospholipids (PC, PE, LPC, PI, PA and PS) changes in dependence on cultivation temperature. In all 8 strains the content of PE decreases and that of PI rises with increasing temperature. The temperature dependence in other classes of lipids is not clear. For instance, in *C. victoriae*, *C. capitatum* and *H. wattica* the temperature dependence of the content of PC has a maximum at 20 °C. The temperature dependence of PS content in *C. victoriae* and *C. capitatum* has a downward-curved shape, in *H. wattica*, *K. malvinella*, *M. aquatica*, *M. cryoconiti* and *T. aggtelekiense* it is upward-curved. In further experiments we therefore focused primarily on the determination of molecular species of phospholipids that exhibited similar differences in their proportions at different temperatures, i.e. PC, PE, and PI, and also such molecular species at least some of which are commercially available.

3.3. Structure of polar lipids

Phospholipids located in the cells of *K. malvinella* were identified on the basis of the fragmentation mechanism obtained by using mass spectrometry of commercially available standards, see Material and Methods. In negative-ion tandem ESI, mass spectra were used to characterize the structure of each class of phospholipids, as well as the structure of their molecular species, including the regioisomers.

Fragmentation of phospholipids in negative ESI yields mostly deprotonated molecular ion $[M-H]^-$ which in further tandem mass spectrometry yields three main fragment ions representing neutral loss of free fatty carboxylic acid ($[M-H—RCOOH]^-$), $[M-H—R'CH{=}C{=}O]^-$ representing neutral loss of fatty acyl group as a ketene, and fatty carboxylate anion ($[RCOO]^-$). Identification of regioisomers, i.e. the location of the fatty acyl groups at *sn*-1 or *sn*-2 positions on the individual phospholipids can be determined from the relative intensities of $[M-H—R'CHCO]^-$ ions, their relative abundance depending on the regiochemistry.

3.3.1. Phosphatidylcholine (PC)

Since PC contains a quaternary amino group, for its detection in negative ion mode it is necessary to spike the eluate with e.g. acetate buffer; ESI then produces the anion adducts $[M+CH_3COO]^-$ [18]. Upon collision-induced dissociation, anion adducts of PC ions lose both an acetate and a methyl group from the choline moiety to produce a fragment ion (denoted $[M-15]^-$) with *m/z* of 15 Da lower than the mass of the zwitterionic form of intact PC. The $[M-15]^-$ ion undergoes further fragmentation to yield less abundant intermediates. This includes neutral loss of ketene $[M-15—R'CH{=}C{=}O]^-$, a neutral loss of free FA $[M-15—R'CH_2COOH]^-$ and finally generation of acyl anions of FAs $[RCOO]^-$. The acyl anion fragment of *sn*-2 FA was more abundant than the FA at *sn*-1 position. A similar pattern of fragment ions was observed in MS/MS analysis of other glycerophospholipids.

Fig. 2S documents the tandem MS spectrum of a synthetic *sn*-OP-PC standard. Phosphatidylcholine was detected as acetate anions at *m/z* 818.5917 and its spectrum showed major peaks at *m/z* 744.5549 ($[M-15]^-$), 281.2484 ($[C_{17}H_{33}COO]^-$), and 255.2329 ($[C_{15}H_{31}COO]^-$). Ions with much lower abundance, which nevertheless sufficed for determining the structure, included ions at *m/z* 480.3094 $[M-15—C_{16}H_{31}CH{=}C{=}O]^-$ and at *m/z* 506.3249 $[M-15—C_{14}H_{29}CH{=}C{=}O]^-$ formed by loss of FAs as ketenes, or ions arising by neutral loss of free FA at *m/z* 462.2989 $[M-15—C_{16}H_{31}CH_2COOH]^-$ and at *m/z* 488.3144 $[M-15—C_{14}H_{29}CH_2COOH]^-$. Other ions present in the spectrum, i.e. ions at 673.4812 Da (loss of choline and acetate from precursor ion), at 224.0689 Da (glycerophosphocholine with loss of CH_3 and H_2O), at 168.0429 Da (phosphocholine with loss of CH_3), and at 152.9961 Da (glycerol-3-phosphate ion with loss of H_2O) fully support the proposed structure of *sn*-OP-PC.

3.3.2. Phosphatidic acid (PA)

In negative ESI, PA molecular species with palmitic and oleic acids produce, in addition to an $[M-H]^-$ ion at 673.4814 Da, also four abundant ions. The first of them at 435.2517 Da arises by loss of *sn*-1 acyl chain as ketene ($R'CH{=}C{=}O$) from $[M-H]^-$, another at 417.2412 Da by neutral loss of *sn*-1 RCOOH group from $[M-H]^-$. A similar loss of *sn*-2 acyl chain as ketene ($R'CH{=}C{=}O$) from $[M-H]^-$ produces an ion at 409.2361 Da and neutral loss of RCOOH at *sn*-2 position from $[M-H]^-$, yields an ion at 391.2255 Da. We determined the regioisomers of individual molecular species of PA based on the above mentioned rule on the preferential loss of FA from *sn*-1. The higher abundance of the $[M-H—R_2COOH]^-$ ion at *m/z* 391.2255 than the $[M-H—R_1COOH]^-$ ion at *m/z* 417.2412 and a higher abundance of the $[M-H—R'_2CH{=}C{=}O]^-$ ion at *m/z* 409.2361 than the $[M-H—R'_1CH{=}C{=}O]^-$ ion at *m/z* 435.2517 fully confirmed the structure of PO-PA.

3.3.3. Phosphatidylserine (PS)

In negative ESI, PS containing palmitic and oleic acids, produces the $[M-H]^-$ ion at *m/z* 760.5134 along with the $[M-H-87]^-$ ion at *m/z* 673.4814, which is formed by loss of serine from precursor ion. The

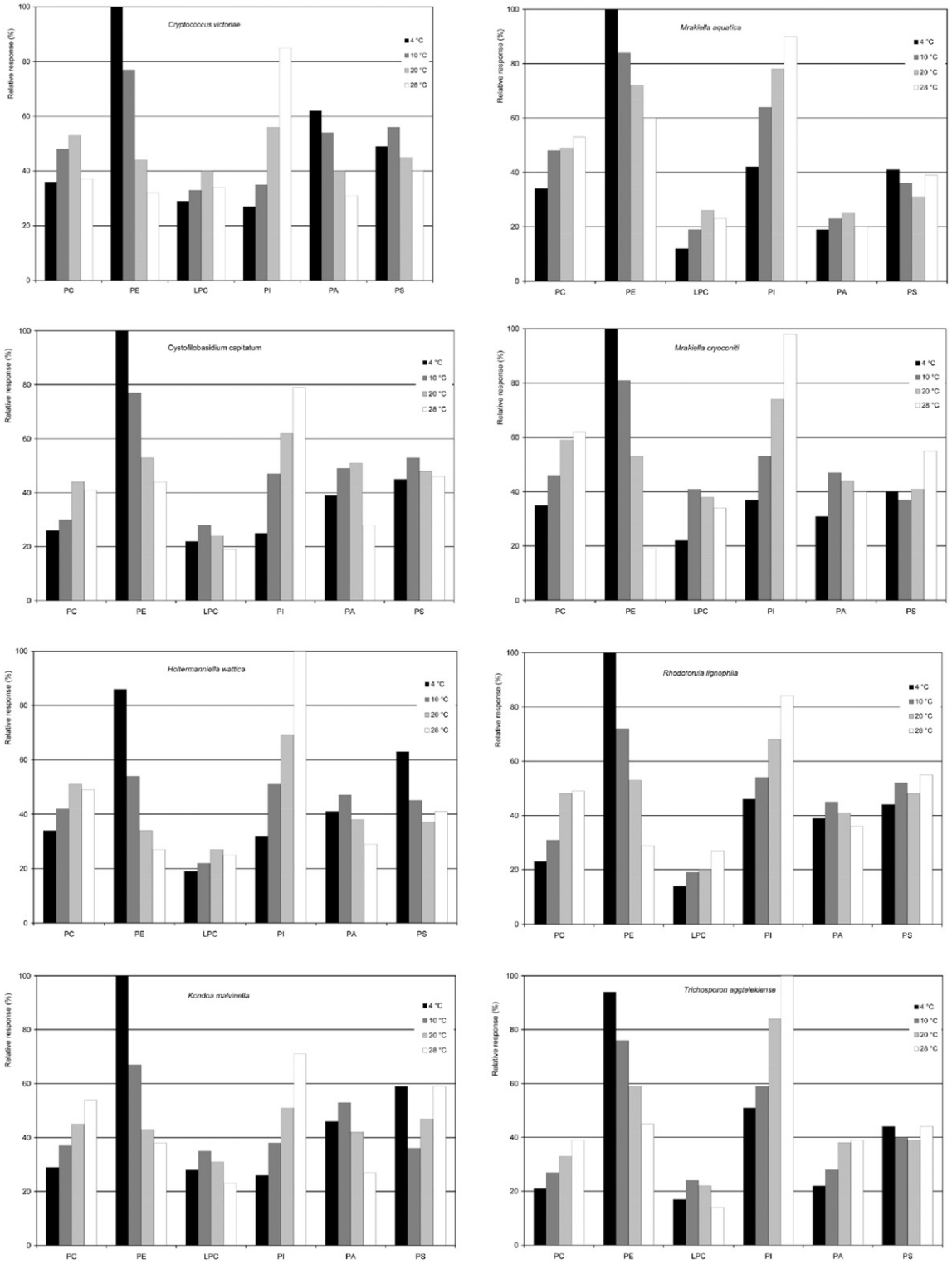

Fig. 3. a–h. Changes in the content of major phospholipids (phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA)) in the eight yeast strains cultivated at different temperatures (4 °C, 10 °C, 20 °C, and 28 °C).

tandem MS spectrum is very similar to the spectrum of PA since the ion at *m/z* 673.4814 is analogous to the $[M-H]^-$ ion of PO-PA. It is therefore not surprising that the abundances of six ions (at *m/z* 435.2517 - loss of *sn*-1 acyl chain as ketene (R′CH═C═O) and serine from $[M-H]^-$, at *m/z* 417.2412 - neutral loss of *sn*-1 RCOOH group and serine from $[M-H]^-$, at *m/z* 409.2361 - loss of *sn*-2 acyl chain as ketene (R′CH═C═O) and serine from $[M-H]^-$, at *m/z* 391.2255 - neutral loss of *sn*-2 RCOOH group and serine from $[M-H]^-$, at *m/z* 281.2486 - *sn*-2 $[RCOO]^-$ ion and at *m/z* 255.2330 - *sn*-1 $[RCOO]^-$ ion) are nearly identical with the corresponding molecular species of PO-PS.

3.3.4. Phosphatidylethanolamine (PE)

Another three major ion types in addition to $[M-H]^-$ were determined in negative tandem mass spectrometry of PE. They include above all ions arising by neutral loss of ketene ($[M-H-R'CH{=}C{=}O]^-$),

neutral loss of free fatty acid ($[M-H—RCOOH]^-$), and fatty carboxylic acid anion ($[RCOO]^-$). Ions that identify the polar head group were also observed at *m/z* 152.9958 (glycerol-3-phosphate ion with loss of H_2O) and at *m/z* 140.0118 (ethanolamine phosphate ion). Since the above rule states that the abundance of fragment ions is dependent on the position of the fatty acyl groups in glycerol backbone, one can determine individual regioisomers of PE. PO-PE contains ions at *m/z* 478.2939 and 452.2783, corresponding to neutral loss of *sn*-1 acyl chain as ketene ($R'_1CH{=}C{=}O$) from $[M-H]^-$ and loss of *sn*-2 acyl chain as ketene ($R'_2CH{=}C{=}O$) from $[M-H]^-$, respectively. The latter ion is more abundant than the former, suggesting that the pathway leading to the ketene loss at *sn*-2 ($[M-H—R'_2CH{=}C{=}O]^-$) is likely sterically more favorable. Tandem mass spectrum further contains ions at *m/z* 460.2834 and 434.2677, respectively, corresponding to neutral loss of the fatty acid moiety at *sn*-1 ($[M-H—R_1COOH]^-$) and at *sn*-2 ($[M-H—R_2COOH]^-$). Like before, the ion corresponding to the loss of fatty acid at *sn*-2 ($[M-H—R_2COOH]^-$) is slightly more abundant than the ion corresponding to the same loss at *sn*-1 ($[M-H—R_1COOH]^-$). Other ions present in the spectrum include ions at *m/z* 281.2486 ($[RCOO]^-$ from *sn*-2) at *m/z* 255.2330 ($[RCOO]^-$ from *sn*-1) at *m/z* 152.9958 (glycerol-3-phosphate ion with loss of H_2O), and at *m/z* 140.0118 (ethanolamine phosphate ion). Other molecular species of PE were determined in a similar way.

3.3.5. Phosphatidylinositol (PI)

In negative ESI, MS spectrum of PI containing again palmitic and oleic acids displays abundant $[M-H]^-$ ion at *m/z* 835.5342. Tandem mass spectrum features major fragment ions of three groups, viz. a pair formed by neutral loss of RCOOH group ($[M-H—RCOOH]^-$), loss of acyl chain as ketene ($R'CH{=}C{=}O$) from $[M-H]^-$, and fatty carboxylate anions, i.e. $[RCOO]^-$. Other ions present are derived from inositol polar head group at *m/z* 673.4814 (loss of inositol from $[M-H]^-$), at *m/z* 315.0487 and 297.0381, respectively (glycerophosphoinositol with loss of one or two H_2O). The tandem mass spectrum $[M-H]^-$ ion of PO-PI at *m/z* 835.5342 contains ions at *m/z* 579.2940 and 553.2783, representing neutral loss of RCOOH group from $[M-H]^-$. Neutral loss of the *sn*-1 acyl chain as ketene ($R'CH{=}C{=}O$) or *sn*-2 acyl chain as ketene ($R'CH{=}C{=}O$) yields an ion at *m/z* 597.3045 ($[M-H—R'_1CH{=}C{=}O]^-$) or 571.2889 ($[M-H—R'_2CH{=}C{=}O]^-$), respectively. These results are similar to that observed for PA, see above. Four other ions also present are formed similarly to ions $[M-H—RCOOH]^-$ and $[M-H—R'CH{=}C{=}O]^-$ and to loss of inositol, e.g. ion at *m/z* 435.2517 (loss of *sn*-1 acyl chain as ketene ($R'CH{=}C{=}O$) and inositol from $[M-H]^-$), etc.

3.3.6. Lysophosphatidylcholine (LPC)

The molecular species of lysophosphatidylcholine (e.g. *sn*-1 P-LPC) under negative ESI provide spectra very much like those of PC, the most abundant being demethylated ion ($[M-CH_3]^-$) at *m/z* 480.3096. Ion at *m/z* 554.3464 $[M+CH_3COO]^-$ is present in minor proportion. Highly abundant is the ion at *m/z* 255.2330 ($[RCOO]^-$). Four minor ions include ions at *m/z* 242.0799 due to loss of *sn*-1 acyl chain as ketene ($RCH{=}C{=}O$), CH_3 and acetate from precursor ion, or ion at *m/z* 224.0693 formed by neutral loss of *sn*-1 RCOOH, CH_3 and acetate from precursor ion.

The analysis of the above phospholipids in *K. malvinella* as shown in Figs. 3 and 1S was followed by their quantification. Fig. 2S depicts mass spectra of two commercially available standards (*sn*-OP-PC and *sn*-PO-PC) and the same molecular species from the yeast *K. malvinella* cultivated at four different temperatures (4 °C, 10 °C, 20 °C, and 28 °C). As seen in the mass spectra, the abundances of four ions, i.e. ions at *m/z* 480.3094 $[M-15—C_{16}H_{31}CH{=}C{=}O]^-$ and at *m/z* 506.3249 $[M-15—C_{14}H_{29}CH{=}C{=}O]^-$ and another pair at *m/z* 462.2989 $[M-15—C_{16}H_{31}CH_2COOH]^-$ and at *m/z* 488.3144 $[M-15—C_{14}H_{29}CH_2COOH]^-$ differ, or in other words the proportion of OP-PC having phase transition temperatures −9 °C gradually changes with increasing cultivation temperature to PO-PC having phase transition temperature −2 °C. This phenomenon is clearly illustrated in the cutout from mass spectra, see Fig. 3S.

In further analysis we again focused on the content of regioisomers of PE containing oleic and palmitic acids. It is one of the few PE with two different FA that are found in yeast and is also commercially available in both regioisomers. When analyzing PE (see above and Figs. 4S and 5S) (cutoff of Fig. 4S), it was found that the ratio of molecular species, i.e. PO-PE and/or OP-PE changes with the cultivation temperature.

Major molecular species of phospholipids of the yeast *K. malvinella* were further analyzed by negative ESI tandem MS. Tables 2 and 3 clearly reflects the effect of temperature on the proportion of individual molecular species. This is documented, e.g., by the nearly 100-fold drop of LL-PC molecular species with increasing cultivation temperature. When we note that the phase transition temperature of LL-PC is −53 °C, it is a completely understandable phenomenon.

Conversely, the content of PP-PC, which has a phase transition temperature of 41 °C, increased at 28 °C more than twice as compared with 4 °C.

All other 7 strains of psychrophilic yeast behaved in a similar manner, see Tables 2S, 3S, and 4S which give the values for the three major phospholipids, i.e. PC, PE and PI. Thus, like Table 2, this table shows the effect of cultivation temperature on the content of the molecular species of each phospholipid class. In accordance with the content of fatty acids of phospholipids, see Table 1S, one can note a decrease of molecular species containing linoleic and linolenic acids, and an increase of molecular species containing saturated FA (P and S).

4. Discussion

4.1. Fatty acid regulation in response to growth temperature

Fluid properties of cell membranes are significantly influenced by the proportion of acyls in fatty phospholipid bilayers. Microorganisms which are capable of growth in a broad temperature range can adapt the composition of phospholipid bilayer to current growth conditions. Increasing number of double bonds in the acyl chain of the fatty acid ensures a high fluidity of the membrane even in unfavorable culture conditions of low temperature; without this adjustment membrane fluidity would be disturbed [2,19]. Rossi et al. [3] observed a significant increase in the percentage of PUFA in psychrophilic yeast at 4 °C accompanied by a decrease of saturated FA. We also observed a similar trend: a significant increase of PUFA associated with a simultaneous decrease of MUFA at 4 °C was observed in all eight strains of yeast, see Fig. 1 and Tables 1 and 1S. Recent studies indicate that high levels of α-linolenic acid in cells cultured at 5 °C could reflect the cell's effort to maintain optimal membrane fluidity at low temperature [20]. Our results show that the highest content of α-linolenic acid in the biomass was at 4 °C. Rossi et al. [3] also reported that in obligately psychrophilic yeast strains the share of α-linolenic acid can amount to 37% of the total fatty acids. The yeast species in our study contained up to 20% α-linolenic acid in the biomass. Even yeast genera which lack the ability to form highly unsaturated fatty acids are capable of increasing the total content of unsaturated fatty acid at low culture temperatures [21]. Our results indicate that the yeast species under study were able to grow over a wide range of temperatures (4–28 °C). Because the selected yeasts were from the DBVPG Collection, in which they are included among psychrophilic yeast, they reached the highest increase in biomass at low culture temperatures (4 or 10 °C). The highest monitored temperature of 28 °C was lethal for some of them (*M. aquatica*, *M. cryoconiti*, *C. capitatum*). A similar finding was made by Branda et al. [22] who did not notice any

Table 2
Lipidomics of *K. malvinella* grown at different temperatures.

	Mol spec PC				Mol spec PE				Mol spec PI				Mol spec PG				Mol spec PS			
	4 °C	10 °C	20 °C	28 °C	4 °C	10 °C	20 °C	28 °C	4 °C	10 °C	20 °C	28 °C	4 °C	10 °C	20 °C	28 °C	4 °C	10 °C	20 °C	28 °C
15:0/L	0.7	0.5	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15:0/Po	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/L	0.5	0.5	0.3	0.2	0.5	0.5	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/O	0.4	0.6	0.6	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B/L	0.5	0.5	0.2	0.1	0.5	0.4	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B/O	0.0	0.0	0.0	0.0	0.4	0.4	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.7	0.6	0.8
E/L	0.0	0.0	0.0	0.0	0.5	0.4	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L/L	18.0	8.8	3.3	0.8	17.7	10.5	3.4	0.8	0.0	0.0	0.0	0.0	24.3	17.9	5.6	1.5	0.0	0.0	0.0	0.0
Ln/L	7.2	3.9	1.3	0.4	8.3	5.2	1.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ln/Ln	0.0	0.0	0.0	0.0	4.6	2.6	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/L + Po/Po	1.2	0.8	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/M	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/Mo	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/O	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.1	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/P	0.9	0.8	0.6	0.6	0.0	0.0	0.0	0.0	1.4	1.0	0.7	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/Po	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1
Ma/G	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ma/L	0.7	0.6	0.3	0.1	0.7	0.6	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ma/O	0.5	0.7	0.5	0.5	0.5	0.6	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ma/P	0.5	0.7	0.7	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mo/L	0.6	0.5	0.2	0.1	0.6	0.5	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mo/O	0.4	0.6	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mo/P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.8	0.7	0.7	0.0	0.0	0.0	0.0	1.5	1.0	0.7	1.0
Mo/Po	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mo/S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O/B	0.4	0.5	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O/E	0.0	0.0	0.0	0.0	0.4	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O/E + G/G	0.4	0.6	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O/G	0.0	0.0	0.0	0.0	0.4	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.9	0.6	0.8
O/L	11.9	8.3	6.4	3.0	13.0	11.5	6.6	3.1	21.2	16.3	7.7	4.5	19.2	21.4	10.9	5.8	26.9	19.3	7.9	4.9
O/Lg	0.4	0.5	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O/Ne	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.9	0.7	0.9
O/O	9.8	14.6	12.4	11.5	9.6	12.5	12.8	12.1	15.7	18.1	14.9	10.1	18.0	18.8	26.7	22.3	19.7	21.3	15.3	11.0
O/S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	2.7	5.5	8.9
P/15:0	0.5	0.6	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P/G	0.0	0.0	0.0	0.0	0.4	0.5	0.6	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P/L	13.8	8.9	8.0	4.5	12.9	10.8	8.3	4.7	18.9	15.5	9.6	6.8	22.6	15.1	13.7	8.7	0.0	0.0	0.0	0.0
P/Ln	5.2	6.2	3.1	2.4	5.1	5.3	3.2	2.5	11.6	7.7	3.7	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P/Mo + 15:0/O	0.9	1.2	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P/O	9.7	13.7	19.6	17.2	9.4	11.8	20.3	18.1	15.6	17.0	23.6	21.3	10.1	13.7	22.1	33.3	19.6	19.8	24.4	16.6
P/P	9.2	9.6	15.0	25.7	9.5	11.1	18.7	27.0	5.4	9.1	19.8	26.1	0.0	0.0	0.0	0.0	19.5	18.6	22.9	28.2
P/Po	0.0	0.0	0.0	0.0	0.9	1.2	3.0	4.5	1.5	1.7	3.5	6.5	1.7	2.0	4.9	8.3	1.8	1.9	3.6	7.1
P/Po + M/O	0.9	1.3	2.9	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P/S	0.5	2.4	5.6	8.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Po/L	1.2	1.3	1.2	0.7	1.2	1.1	1.2	0.8	2.0	1.6	1.4	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Po/Ln	0.5	0.6	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Po/O	0.9	1.4	2.3	2.9	0.9	1.2	2.4	3.0	1.5	1.8	2.7	4.3	1.7	2.1	3.9	5.6	1.8	2.1	2.8	4.7
Po/Po	0.1	0.1	0.4	0.7	0.0	0.0	0.0	0.0	0.1	0.2	0.5	1.1	0.1	0.2	0.7	1.4	3.1	1.4	0.9	1.2
S/A + P/B	0.0	0.1	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S/G	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.4
S/G + P/E	0.0	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S/L	0.7	3.5	2.3	1.4	0.7	3.0	2.4	1.5	1.2	4.4	4.0	2.1	1.3	5.2	3.9	2.7	1.4	5.1	7.3	2.3
S/Ln	0.3	1.7	0.9	0.7	0.3	1.5	0.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S/O	0.5	2.3	5.9	5.4	0.5	1.7	3.7	5.7	0.8	2.8	5.3	8.2	1.0	3.6	7.6	10.4	1.1	3.5	5.5	8.9
S/P	0.0	0.0	0.0	0.0	0.5	3.1	5.8	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S/Po	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	1.0	2.0	0.0	0.0	0.0	0.0	0.1	0.5	1.0	2.2
S/S	0.0	0.0	0.0	0.0	0.0	0.9	1.6	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S/S + A/P	0.0	1.0	1.6	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

growth of certain yeast at 30 °C. The content of total lipids in the monitored strains was mostly in the range of 0.5 to 2.9% except for *T. aggtelekiense* and *M. cryoconiti* in which it ranged from 3.1 to 10.0%. Similar values of total lipids were reported by Libkind [23], who cultivated psychrophilic yeast from Patagonia. In general, Fig. 1 indicates that with decreasing temperature the saturated fatty acid content decreases and that of polyunsaturated fatty acids increases. An exception is oleic acid, for which the temperature dependence of its content is species specific, see the studies [20,21,24] and our published results, predominantly see Fig. 2.

4.2. Polar lipids

As early as 30 years ago some authors [25] have noted the possibility of adaptation of biological membranes to temperature via a change of the content of molecular species of PC and PE in mitochondrial and microsomal membranes of liver from thermally-acclimated rainbow trout. This finding led to the formulation of four strategies of adaptation of membranes to changes of environmental and physiological conditions including temperature. These strategies include above all "changes in the acyl chain and polar head group structures, and reshuffling of acyl

Table 3
Lipidomics of *K. malvinella* grown at different temperatures - lysophosphatidylcholine.

Mol spec LPC	4 °C	10 °C	20 °C	28 °C
M	1.0	0.7	0.1	0.0
14:1	1.3	0.4	0.1	0.0
15:0	0.3	0.2	0.0	0.0
P	24.3	25.5	36.4	42.0
Po	1.4	1.8	4.5	6.2
Ma	0.4	0.4	0.3	0.3
Mo	0.2	0.2	0.1	0.1
S	0.4	6.5	9.7	12.5
O	24.4	27.2	28.7	27.8
L	33.5	24.8	14.3	6.5
Ln	12.7	11.8	4.9	3.0
A	0.0	0.2	0.4	0.7
G	0.1	0.2	0.2	0.3
B	0.0	0.0	0.1	0.2
E	0.0	0.1	0.1	0.2
Lg	0.0	0.0	0.1	0.2

chains to form new lipid molecular species without changing the average acyl chain composition" [26]. As yet, the analytical techniques have not made it possible to verify this theory, i.e. to determine individual molecular species including regioisomers of phospholipids. By using lipidomic analysis [18], the studies performed by the Shevchenko group [10,11] have enabled the identification of both lipid classes and corresponding molecular species in the yeast *Saccharomyces cerevisiae*. Though the authors [11] used only two different cultivation temperatures (24 °C and 37 °C), their results confirmed that an increase in cultivation temperature is accompanied by an increase in the content of PI and a drop in the content of PE. The other study [10], performed also on *S. cerevisiae* strains and recording also the content of lipid classes and molecular species at four different cultivation temperatures, showed that in four lipid classes (PA, PC, LPC and PS) the temperature dependence is not very noticeable while in PE and PI it is clear-cut, the content of PI increasing and that of PE decreasing with increasing temperature.

Another study on *S. cerevisiae* showed that strains cultivated at 35 °C contained higher amounts of PI that those cultivated at 15 °C, the trend with PE and PC being opposite [27] and endeavored to explain the reasons for the changes in the polar head group structures. We assume that the following factors may play a role in this phenomenon. First and foremost, it is the volume of the molecule of the relevant phospholipid. This volume depends mainly on the head-group. Molecular volumes for lipids composed of palmitoyl-oleoyl (PO) chains and different head groups are different and depend on the temperature. With increasing temperature, the molecular volume of the phospholipid increased [28]. At 25 °C, the volume of PO-PC is around 1250 A^3 while PO-PE does not attain the same volume even at 60 °C, reaching only 1200 A^3.

Another related factor affecting the molecular shape, which holds for all the phospholipids in general, is the type of acyl chains. Thus PC species bearing two saturated fatty acyl chains will occupy a different space than the species having two unsaturated FA. A change of unsaturation, e.g. from palmitoleate to palmitate, induces a shift from a rather conical to a cylindrical shape [29].

As described in Results, we analyzed molecular species of major phospholipids, viz. Fig. 3a–h. Unfortunately, our data on the proportion of individual molecular species can be compared with the results of other studies only with difficulty because of the paucity of data in the latter. Thus Bhuiyan et al. [30] analyzed phospholipid molecular species from Antarctic yeasts but their analysis of PC content in *H. wattica* cultivated at 5 °C identified only three molecular species (PoO-PC, LL-PC, and OL-PC), i.e. too few to enable comparison with cultivation at 15 °C. We identified in the same species, though from a different collection, more than 40 molecular species. Even so, some comparison of the trends can be done. With increasing temperature the content of PoO-PC rises while that of LL-PC decreases. In our study, the drop in the content of LL-PC was nearly 100-fold while the content of PoO-PC increased more than twice.

As mentioned above and illustrated in Figs. 4S and 5S, a change in temperature brings about, apart from PC (molecular species PO-PC versus OP-PC), changes in analogous molecular species of PE, i.e. PO-PE and OP-PE. It has been reported that the phase transition temperature of PO-PC differs from OP-PC by 7 °C [31] and the difference between PO-PE versus OP-PE is still greater, some 10 °C [32]. Based on these temperature differences, we assume that the cell compensates for the adverse temperature effect by changing the fatty acids in the *sn*-1 and *sn*-2 positions.

Recent studies of yeast lipidome [10] and regulation of membrane phospholipids in yeast [33] concern solely the technologically important *Saccharomyces cerevisiae*; other yeast species have not been explored save for a single study [30], which used tandem ESI for analyzing both Antarctic and non-Antarctic yeasts cultivated at different temperatures. Yeast phospholipids were found to prefer unsaturated FA at *sn*-2 position and the content of unsaturated FA was found to be higher at lower temperature.

5. Conclusion

Microorganisms regulate the composition of cell membranes in order to maintain homeostasis and constant optimal membrane viscosity [34]. Membrane fluidity depends on, e.g. the ratio of saturated to unsaturated fatty acids. To maintain membrane fluidity at lowered temperature, the cells respond by initializing the slower de novo synthesis of phospholipids and/or by the faster upregulation of enzymes that remodel membranes by (a) desaturating single bonds of fatty acyl chains to form double bonds, (b) reshuffling the chains between different phospholipid molecules to produce ones that contain two unsaturated fatty acids, (c) changing the length of the acyl chains in membrane lipids [35] or (d) changing the head groups of the phospholipids [36].

Another relatively fast membrane effect is a change in the PC/PE-ratio, which is decreased when the temperature decreases. The reason for this decrease can be de novo synthesis of PE, decarboxylation of PS to PE and/or methylation of PE to PC [37]. PE tends to decrease the order of a lipid bilayer as it prefers a conical rather than a cylindrical geometry [37]. The small head group size and anionic character of PE can allow its interaction with small molecules (e.g. small sugars) that increase membrane fluidity.

Conflict of interest

The authors declare that they have no conflict of interest. All the authors agree with the submission of the manuscript to BBA-Lipids. The research does not involve any human participants and/or animals.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbalip.2016.07.005.

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