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ORIGINAL PAPER

Influencing fatty acid composition of yeasts by lanthanides

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Abstract The growth of microorganisms is affected by cultivation conditions, concentration of carbon and nitrogen sources and the presence of trace elements. One of the new possibilities of influencing the production of cell mass or lipids is the use of lanthanides. Lanthanides are biologically non-essential elements with wide applications in technology and industry and their concentration as environmental contaminants is therefore increasing. Although non-essential, lanthanides have been proposed (and even used) to produce beneficial effects in plants but their mechanisms of action are unclear. Recently, it was suggested that they may replace essential elements or operate as potent blockers of Ca^{2+} channels. We tested the effect of low concentrations of lanthanides on traditional biotechnologically useful yeast species (*Kluyveromyces polysporus, Saccharomyces cerevisiae, Torulospora delbrueckii*), and species capable of high accumulation of lipids (*Rhodotorula glutinis, Trichosporon cutaneum, Candida* sp., *Yarrowia lipolytica*). Low concentrations of lanthanum and monazite were conducive to an increase in cell mass and lipids and also higher production of palmitoleic acid, commonly used in cosmetics and medicine, and ω6-linoleic acid which is a precursor of thromboxanes, prostaglandins and leucotrienes.

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Introduction

Heterotrophic microorganisms, such as yeasts, bacteria and fungi have a great potential for applications in biotechnology due to their high growth rate and the possibilities of industrial mass cultivation in suspension cultures which are technically easy to control.

Cultivation of yeast takes place in nutrient solutions, which must contain all the elements necessary for cell growth such as P, N, Mg, K and others, including trace elements (Mo, Cu, Zn, etc.). There are dozens of different prescriptions on the composition of nutrient media. The basic source of nutrients for heterotrophic microorganisms is carbon, which comes primarily as an organic compound such as glucose or cheaper non-traditional substrates or waste materials such as whey and molasses or volatile fatty acids (du Preez et al. 1995, 1997; Fei et al. 2011; Chang et al. 2010).

Various stimulants are being currently explored to accelerate the growth of microorganisms and increase the production of cell mass and biotechnologically useful compounds. Among these are, e.g., phytohormones and various other chemicals, including methanol, ethanol, spermidine or even local anesthetics. Another possibility is to use mixed cultures with other bacteria, yeast or fungi. Some of these possibilities are effective but they also entail various drawbacks such as increased risk of bacterial contamination, the necessity of producing the stimulant by chemical synthesis, adverse effect of the stimulant on other organisms after its release into the environment, or high cost.

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Rare earth elements, which are known as non-essential elements include scandium, yttrium, lanthanum and the series of 14 lanthanides with atomic numbers 58–71. These are members of group IIIb (f-block) in the periodic table, and hence exhibit very similar physical and chemical properties due to the lanthanide contraction. These elements are widespread in nature and are generally available in terrestrial and marine environments, but rarely occur in concentrated forms (Brown et al. 1990). Currently, due to their unique physical and chemical properties, they are used in a growing number of various sectors (Du and Graedel 2011). The potential importance of lanthanides in biology and agriculture is a rather contradictory matter (Tyler 2004), though mixtures of lanthanide compounds have been widely used in past decades in Chinese agriculture to increase crop yields (Pang et al. 2002; Xu et al. 2002). Like other trace elements, lanthanides show both a positive and a negative impact on plant growth and development at low and high concentrations (Goecke et al. 2015).

Although lanthanides were not confirmed as elements essential for plant growth, studies on various crops (tobacco, tomatoes, wheat) have shown their positive effects on growth and quality of the crops (Hu et al. 2004; Pang et al. 2002; Tyler 2004; Volland et al. 2014). Lanthanides influence the chemical composition of living organisms and their content of substances such as lipids and pigments. It has already been shown that the addition of La^{3+} and Ce^{3+} brings about an increase in the concentration of polar and non-polar lipids in cell membranes (Xing and Weng 1991). La^{3+} ions also increase the content of unsaturated fatty acids (Li et al. 1992).

In the long term, the effects of lanthanides on heterotrophic organisms have been poorly explored. Generally, these effects were studied rather to determine their toxicity and accumulation in the cell mass, their toxicity during their production and processing in the steelworks or as undesirable contamination of mine water (Das and Das 2013; Evseeva et al. 2010; Jin et al. 2009; Oliveira et al. 2012). The salts of rare earth elements in certain concentrations have been found to stimulate the growth of certain heterotrophs; however, specific mechanisms of such growth promoting action are still unknown. For instance, hardly any data have been published on the effect of lanthanides on the fatty acid profile in yeast. The aim of the present publication is to explore their potential activity in stimulating the growth of heterotrophic microorganisms, especially both oleaginous (*Rhodotorula glutinis*, *Trichosporon cutaneum*, *Candida* sp., *Yarrowia lipolytica*) and non-oleaginous (*Kluyveromyces polysporus*, *Saccharomyces cerevisiae*, *Torulospora delbrueckii*) yeasts for the biotechnological utilization of cell mass, oils and other metabolites.

Materials and methods

Microorganisms

The yeast strains used in the present study were *Candida* sp. DBM 2163; *K. polysporus* DBM 2171; *R. glutinis* CCY 20-2-20; *S. cerevisiae* DBM 2115; *T. delbrueckii* DBM 39; *T. cutaneum* CCY 30-5-10; *Y. lipolytica* CCY 29-26-36 supplied by the Culture Collection of Yeast, Institute of Chemistry, Slovak Academy of Sciences, Bratislava and by the Collection of Yeasts and Industrial Microorganisms of University of Chemistry and Technology, Prague. For long term storage the stock cultures were maintained in 20 % glycerol at −60 °C. Malt extract agar (23 g/L, pH 7) was employed for short term storage.

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 on a rotary shaker at 150 rpm at 28 °C to the late exponential growth phase. For lipid production, 200 mL 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 28 °C in triplicate parallels.

Growth characteristics were determined in the above yeast strains cultivated with different lanthanides or monazite (calculated to lanthanum proportional content) concentrations (0.03; 0.3; 1; 3 mg/L) in the Bioscreen C (Labsystems, Finland) device in microtiter plates. Lanthanide proportion in monazite is depicted in Table 1. Cultivation temperature was 30 °C, optical density was determined every 2 h. All experiments were performed five times. For fatty acid composition analysis, cultivations with praseodymium, lanthanum, gadolinium and monazite in concentration 0.3 or 3.0 mg/L were carried out in 200 mL YPD medium with 6 mL inoculum, at 30 °C on an

Table 1 Proportion (%) of lanthanides in monazite

Lanthanides	Content (%)
La	18.439
Ce	37.925
Pr	4.211
Nd	14.774
Gd	2.937
Th	5.875
Y	9.714
Fe	3.176
Sm	2.943
Sc	0.005

orbital shaker (150 rpm). The cultivations were carried out for 96 h (until early stationary phase). After cultivation, the cells were centrifuged (9000 g, 10 min) and washed two times. Cell mass yield was determined as dry cell weight. Cell mass was frozen at −75 °C and lyophilized.

Lipid extraction

Lyophilized yeast was mixed with 2 mL of 0.1 mol/L Na_2CO_3 and the mixture was briefly ground with ballotini glass beads (diameter 0.2 mm) in a mortar, overlaid with liquid nitrogen and ground again. This process was repeated 3 times and 50 mL of 0.1 mol/L Na_2CO_3 was finally added. The crushed yeast was extracted with a chloroform–methanol mixture according to (Bligh and Dyer 1959). The sample was centrifuged and the lower phase was evaporated to dryness and the lipid dry weight was determined.

Analysis of fatty acid methylesters

The total lipids (~5 mg) were saponified overnight in 10 % KOH–MeOH at room temperature. A fatty acid fraction obtained from the saponification was partitioned between alkali solution (pH 9) and diethyl-ether to remove basic and neutral components. The aqueous phase containing fatty acids was acidified to pH 2 and extracted with hexane. The fatty acid fraction was methylated using BF_3/MeOH (14 % solution of BF_3 from Sigma-Aldrich).

Gas chromatography–mass spectrometry of FAME was done on a GC–MS system consisting of Varian 450-GC (Varian BV, Middelburg, The Netherlands), Varian 240-MS ion trap detector with electron ionization (EI), and CombiPal autosampler (CTC, USA) equipped with split/splitless injector. A SP-2380 column (Supelco) (100 m, 0.25 mm ID, 0.20 μm film thickness) was used for separation. The temperature program started at 60 °C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 160 °C at a rate of 25 °C min^{-1}. The second temperature ramp was up to 220 °C at a rate of 1.0 °C min^{-1}, this temperature being maintained for 10 min. The solvent delay time was set to 8 min. The transfer line temperature was set to 280 °C. Mass spectra were recorded at 3 scans s^{-1} under electron ionization at 70 eV, mass range *m/z* 50–600. FAMEs (fatty acid methyl esters) were identified according to their mass spectra and using a mixture of chemical standards obtained from Sigma-Aldrich.

Statistical analysis was performed with SigmaStat 3.5 (USA). The statistical significance of differences in mean values of the different measured parameters was calculated by one-way ANOVAs and compared with the Tukey's test at the 5 % level of probability.

Results

Effect of lanthanides on growth and lipid content

The group of lanthanides includes 16 elements—lanthanum, cerium, praseodymium, neodymium, samarium, europium, gadolinium, dysprosium, terbium, holmium, erbium, ytterbium, lutetium, thulium, scandium and yttrium. To determine the effect of different concentrations of lanthanides on growth characteristics, we cultured the 7 yeast strains belonging to oleaginous or technological strains in 9 parallels in the Bioscreen C unit successively with all 16 lanthanides at different concentrations (0.03, 0.3, 1, 3 mg/l) in a microtiter plate. The lanthanides did not have any significant inhibitory effect on yeast growth and some of them even showed a stimulatory effect. These pilot trials served as the basis for selecting 3 lanthanides—lanthanum, praseodymium and gadolinium in concentrations of 0.3 mg/l, and monazite as a naturally occurring mixture of lanthanides (for composition ratio see Table 1) at 0.3 and 3 mg/l based on the content of lanthanum in monazite.

The addition of the lanthanides increased cell mass growth (Fig. 1) of most oleaginous strains (*Candida* sp., *R. glutinis* and *Y. lipolytica*) by 10–20 % while lanthanum enhanced the cell mass of *T. cutaneum* by as much as 150 % and other individual lanthanides in the range of 23 and 57 %. Cultivation with monazite increased the cell mass in *Candida* sp., *R. glutinis* and *T. cutaneum* by 30–40 % and in *Y. lipolytica* by 60 %. The effect of lanthanides on non-oleaginous strains was species-specific. No significant effect on the amount of cell mass was observed in *T. delbrueckii*, the cell mass of *K. polysporus* tended to decrease and *S. cerevisiae* recorded an about 20 % increase of the cell mass content compared to control. These changes in lipid content caused by lanthanides resembled the effect of Zn, Mn or Fe observed by Veen and Lang (2004) or the effect observed when increasing the concentration of carbon substrate e.g. glucose (Braunwald et al. 2013).

The lipid content in oleaginous strains ranged between 20 and 50 % dry cell weight (Fig. 1). In non-oleaginous strains it was much lower—between 4 and 9 %. The increase in the content of lipid versus control was species-specific and there was no link between oleaginous and non-oleaginous strains. A sizable increase in lipid content occurred upon addition of praseodymium and lanthanum to *K. polysporus* (from 5 to 9 %), *Candida* sp. (from 35 to 43 %), *Y. lipolytica* (from 18 to 25 %) and *T. delbrueckii* (from 5 to 7 %). A 10 % increase relative to control was observed with lanthanum and praseodymium in *S. cerevisiae* and *R. glutinis*. A ten-fold higher concentration of monazite (3 mg/l) boosted the increase of lipid content by some 50 % compared to the effect of 0.3 mg/l.

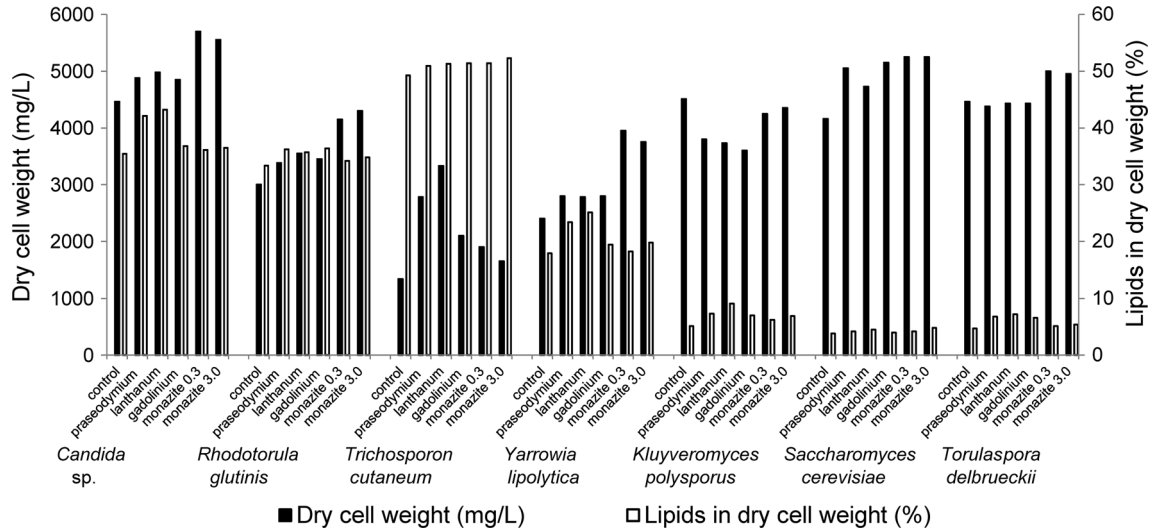

Fig. 1 Cell mass yield (*black bars*—dry cell weight) and lipid content in dry cell weight (*white bars*) of the 7 yeast strains cultured with selected lanthanides and monazite

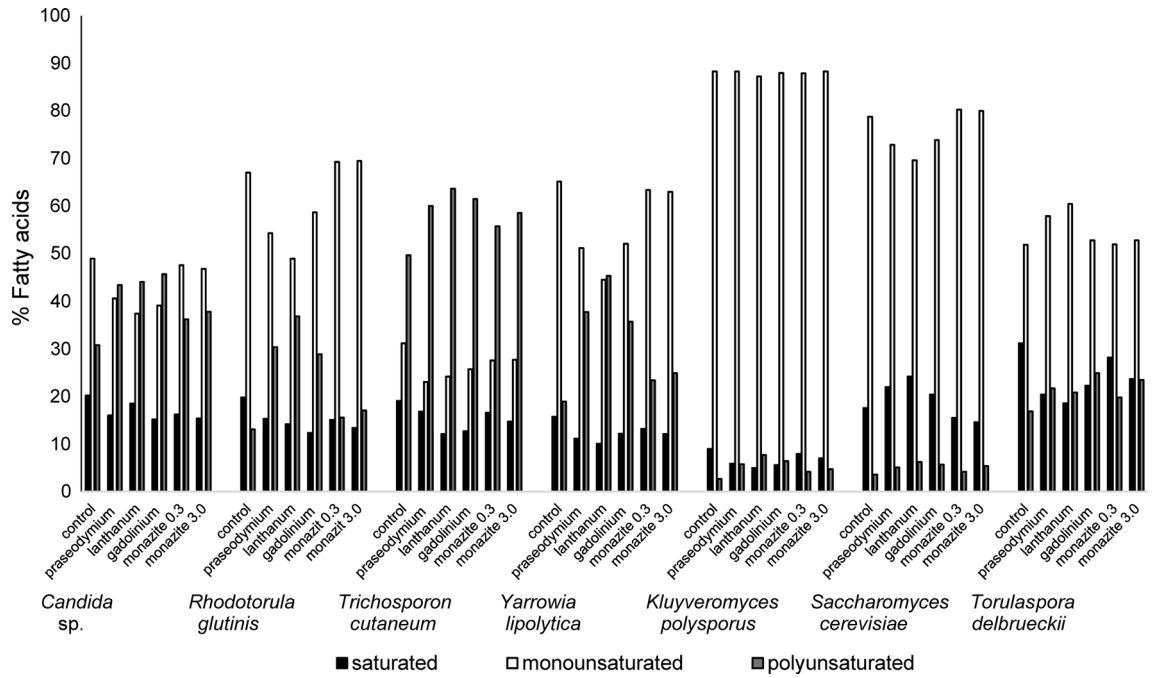

Fig. 2 Proportion (%) of saturated, mono- and polyunsaturated fatty acids in the 7 yeast strains cultivated with selected lanthanides and monazite

Fatty acid composition of lipids

When cultivated with the lanthanides, oleaginous strains showed a 10–18 % content of saturated fatty acids in total FA (Fig. 2), the lowest value being found in *Y. lipolytica* (10.1 % when cultivated with lanthanum). Non-oleaginous yeast showed much higher variability, between 5 and 7 % content of saturated fatty acids in *K. polysporus* in the presence of lanthanides and some 24 % in *T. delbrueckii* and *S. cerevisiae*. All seven strains evinced a decrease relative to the control. A similar situation was with monounsaturated fatty acids. All oleaginous strains plus *K. polysporus* and *S. cerevisiae* showed a decrease of monounsaturated fatty acids in the presence of lanthanum, praseodymium and gadolinium relative to controls and only *T. delbrueckii* increased their proportion from 52 % in the control up to 60.5 % (lanthanum). Cultivation with monazite reached similar values to control in all non-

oleaginous strains and in oleaginous strains except *T. cutaneum*, whereas cultivation with monazite caused a drop in the content of monounsaturated fatty acids from 31.2 % (control) to 27.6 %. The content of polyunsaturated fatty acids increased significantly in all seven strains, the rise ranging from units of % in *K. polysporus* and *S. cerevisiae* to more than 60 % in *T. cutaneum*.

Major fatty acids

Culture conditions affect not only the total lipid content, but they also significantly influence the representation of individual fatty acids (Sitepu et al. 2013). Table 2 shows the changes in the representation of individual FA caused by cultivation with lanthanides. The main fatty acids of oleaginous yeast were oleic acid (28 % in *T.* cutaneum; 67 % in *R. glutinis*) and linoleic acid (50 % in *T. cutaneum*, 13 % in *R. glutinis*), in keeping with literature values (Chen et al. 2013; Sitepu et al. 2013). Cultivation with praseodymium, lanthanum and gadolinium decreased the content of oleic acid by 30 % relative to control in *T. cutaneum* (from 28.6 % in control cultivation to 19.4 % in cultivation with praseodymium). The presence of monazite had no significant impact on the representation of oleic acid in most oleaginous strains, only *T. cutaneum* showing a 15 % decrease against control at both monazite concentrations (from 28.6 to 24.3 %).

In non-oleaginous strains oleic acid is the second most abundant fatty acid (29 % *T. delbrueckii* and *K. polysporus*; 39 % in *S. cerevisiae*) after palmitoleic acid (23 % in *T. delbrueckii*; 59 % in *K. polysporus*). The presence of individual lanthanides (praseodymium, lanthanum and gadolinium) caused a 20–40 % decrease of oleic acid compared to control (e.g. *Candida sp*. from 40.2 to 32.9 % with lanthanum and *S. cerevisiae* from 39.5 to 23.4 % with lanthanum), monazite causing up to 10 % drop (*T. cutaneum, K. polysporus, T. delbrueckii*). In the presence of praseodymium and lanthanum, the content of palmitoleic acid in *T. delbrueckii* increased by nearly 70 % relative to control—from 22.6 to 38.1 %.

Palmitoleic acid and linoleic acid yield

Unlike algae, yeast do not have the enzyme machinery that would enable them to produce DHA acid, but they contain two fatty acids that are nutritionally important—palmitoleic and linoleic acid. Figure 3 shows a summary representation of these fatty acids in oleaginous and non-oleaginous yeasts and their yield (mg/g). A change of culture conditions can lead to increased representation of linoleic acid in oleaginous strains (Sitepu et al. 2013). On expressing the amount of the acids as the yield in mg/g, oleaginous strains contained several times more palmitoleic and linoleic acids than non-oleaginous strains. In terms of the relative proportion of palmitoleic and linoleic acid in fatty acids, the sum of these two fatty acids is usually in all studied strains around 50 %.

It should be noted that the ability of the microbial strains to produce nutritionally important fatty acids depends not only on their relative representation in total fatty acids, but also on the yield of cell mass and lipids in the cell mass. The highest proportion of these two fatty acids in total fatty acids (more than 70 %) among non-oleaginous strains was found in *K. polysporus* in the presence of lanthanum and gadolinium. However, the yield was only about 60 mg/g due to the low content of lipids in the cell (Fig. 1). The wine yeast *T. delbrueckii* contained 46–55 % palmitoleic and linoleic acid in total FA depending on the added lanthanide, and the maximum yield was about 40 mg/g. Although *S. cerevisiae* FA also contained about 50 % palmitoleic and linoleic acid, the yield was only about 20 mg/g due to the lowest lipid content per dry cell weight.

Among the oleaginous strains, the lowest percent proportion and yield of palmitoleic and linoleic acid was recorded in *Y. lipolytica* (up to 52 % and 130 mg/g) and *R. glutinis* (39 % and 135 mg/g). While these two strains showed similar yields, the effect of addition of lanthanides was very different. In *Y. lipolytica*, addition of individual lanthanides increased the relative proportion by 30–50 % relative to the control (from 33 % of total fatty acids to 44.1–52.3 % of total fatty acids), the yield was increased on addition of lanthanum by 122 %. The presence of monazite had no significant effect on either the change of the relative proportion or on the yield in *Y. lipolytica* cultivations. In contrast, *R. glutinis* responded to the presence of lanthanides most of all studied strains. The addition of individual lanthanides increased the proportion of both palmitoleic acid and linoleic by more than 100 % (from 13.3 to 36.9 %) and the yield by more than 140 % (from 44 to 132 mg/g). Addition of monazite (0.3 mg/l) to *R. glutinis* also had a significant effect, increasing both the proportion and the yield by 200 % compared to control (from 13.3 % of total fatty acids to 39.6 % of total fatty acids and from 44 to 135 mg/g). Increasing the concentration of monazite from 0.3 to 3 mg/l had no additional effect on the monitored parameters in *R. glutinis*. *Candida* sp. reached a yield of 200 mg/g palmitoleic and linoleic acid, with the proportion around 50 % of total fatty acids. The addition of lanthanides increased the content of selected fatty acids by tens of percent, addition of monazite increasing the yield by some 10 % (from 140 to 160 mg/g). The yeast that appeared to be most useful for producing palmitoleic and linoleic acid under our conditions with the addition of lanthanides was *T. cutaneum*. The maximum yield of palmitoleic and linoleic acid was 300 mg/g, with the proportion between 59 and 67.2 % of total fatty acids for

Table 2 Proportion (%) of fatty acids in the 7 yeast strains cultivated with selected lanthanides and monazite

Yeast	Lanthanides	(mg/l)	P (%)	Po (%)	S (%)	O (%)	Li (%)	A (%)
Candida sp.	Control	0	17.4	8.8	2.4	40.2	30.8	0.4
	Praseodymium	0.3	12.1	5.3	0.7	35.3	43.4	3.2
	Lanthanum	0.3	14.8	4.5	0.9	32.9	44.1	2.8
	Gadolinium	0.3	11.4	5.9	1.0	33.2	45.7	2.8
	Monazite	0.3	12.6	7.1	1.4	40.5	36.2	2.2
	Monazite	3	12.8	6.5	0.9	40.3	37.8	1.7
Rhodotorula glutinis	Control	0	12.6	0.2	7.2	66.9	13.1	0.0
	Praseodymium	0.3	9.2	0.1	5.4	54.2	30.4	0.7
	Lanthanum	0.3	8.4	0.1	5.3	48.9	36.8	0.5
	Gadolinium	0.3	7.6	0.1	3.9	58.6	28.9	0.9
	Monazite	0.3	9.4	0.1	4.9	69.2	15.6	0.8
	Monazite	3	8.6	0.1	4.4	69.4	17.1	0.4
Trichosporon cutaneum	Control	0	17.3	2.6	1.8	28.6	49.7	0.0
	Praseodymium	0.3	14.9	3.7	1.1	19.4	60.1	0.8
	Lanthanum	0.3	10.3	3.5	0.9	20.7	63.7	0.9
	Gadolinium	0.3	10.9	3.8	1.1	22.0	61.5	0.7
	Monazite	0.3	14.2	3.2	1.0	24.4	55.8	1.4
	Monazite	3	13.0	3.4	0.8	24.3	58.6	0.9
Yarowia lypolytica	Control	0	13.9	14.0	1.2	51.2	19.0	0.7
	Praseodymium	0.3	8.4	9.7	0.6	41.5	37.7	2.1
	Lanthanum	0.3	7.3	6.9	0.5	37.6	45.4	2.3
	Gadolinium	0.3	8.6	8.4	0.8	43.7	35.7	2.8
	Monazite	0.3	10.4	10.8	0.9	52.6	23.4	1.9
	Monazite	3	9.8	10.6	0.7	52.4	24.9	1.6
Kluyveromyces polysporus	Control	0	7.5	59.2	1.0	29.1	2.7	0.5
	Praseodymium	0.3	4.8	65.2	0.7	23.1	5.8	0.4
	Lanthanum	0.3	3.9	64.7	0.9	22.6	7.7	0.2
	Gadolinium	0.3	4.4	66.4	0.8	21.6	6.4	0.4
	Monazite	0.3	6.5	61.4	0.8	26.5	4.2	0.6
	Monazite	3	6.1	61.9	0.4	26.4	4.7	0.5
Saccharomyces cerevisiae	Control	0	11.8	39.3	4.3	39.5	3.6	1.5
	Praseodymium	0.3	17.8	44.8	2.5	28.1	5.1	1.7
	Lanthanum	0.3	20.1	46.2	2.2	23.4	6.2	1.9
	Gadolinium	0.3	17.4	49.7	1.8	24.2	5.7	1.2
	Monazite	0.3	9.5	41.9	2.9	38.4	4.2	3.1
	Monazite	3	8.9	41.8	2.7	38.2	5.4	3.0
Torulaspora delbrueckii	Control	0	26.8	22.6	2.8	29.3	16.9	1.6
	Praseodymium	0.3	14.8	38.1	1.4	19.8	21.7	4.2
	Lanthanum	0.3	13.1	37.4	1.7	23.1	20.9	3.8
	Gadolinium	0.3	15.6	30.3	1.6	22.5	24.9	5.1
	Monazite	0.3	20.1	24.8	2.1	27.2	19.8	6.0
	Monazite	3	18.2	26.2	1.5	26.6	23.5	4.0

lanthanum and monazite in both concentrations. Significantly, the high production of palmitoleic and linoleic acid observed in the presence of lanthanum was similarly high in the presence of monazite, with no significant influence of monazite concentration.

Discussion

Many yeast studies have been focused on exploring the influence of different modes of nutrition—variability of carbon sources (Huang et al. 2013; Chen et al. 2013;

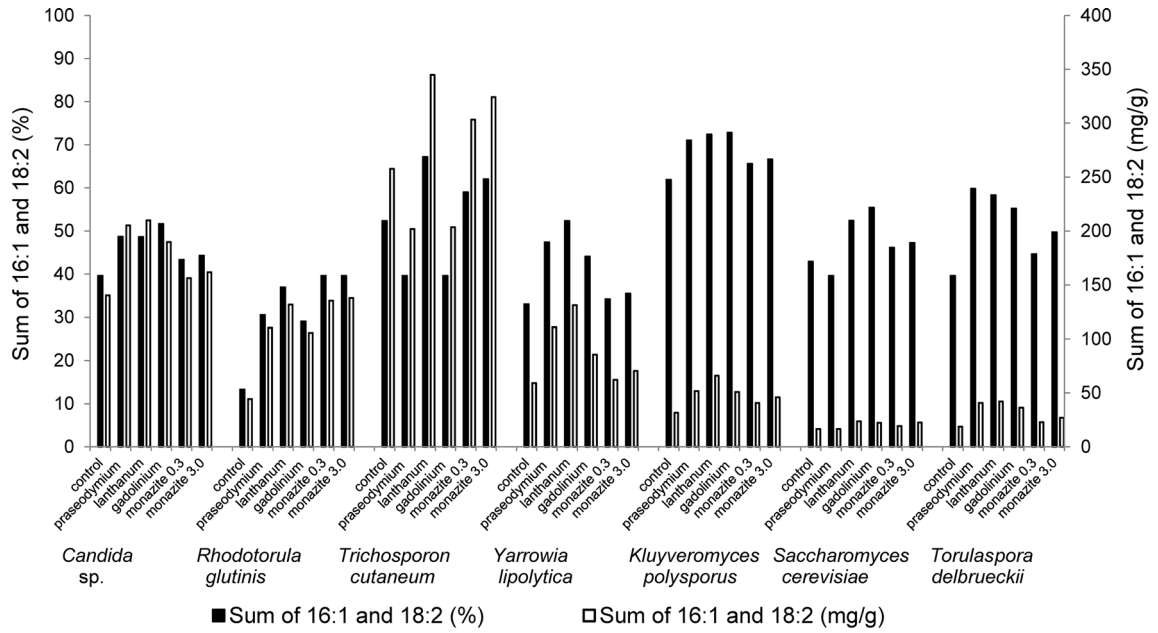

Fig. 3 Content of palmitoleic and linoleic acid in total lipids (%—*black bars*) and the yield of the acids per dry cell weight (mg/g—*white bars*) of the 7 yeast strains cultured with selected lanthanides and monazite

Juszczyk et al. 2013), and the presence of micronutrients and their effect on the lipid composition of cells (Chen et al. 2013). Microbial lipids are considered for possible use as biofuel, are used in animal feed (*S. cerevisiae*), but can also be a source of nutritionally valuable fatty acids such as palmitoleic and linoleic acid. Besides nutritional changes, the composition and lipid content may be also influenced by culture conditions such as pH, temperature, and culture duration (Ageitos et al. 2011; Dey and Maiti 2013; Chen et al. 2013). Our culture conditions—culture temperature of 28 °C, medium pH 6.0 and cultivation period of 96 h—are based on these studies and on our own experience (Kolouchova et al. 2015a, b). Yeast strains were selected either for their ability of a high accumulation of storage lipids, sometimes in excess of 30 % (Dey and Maiti 2013) or on the use of a strain for technological purposes such as brewing, winemaking and the possible use of cell mass as a byproduct (Belda et al. 2015; Rezanka et al. 2013), easy cultivation in large volumes and independence of the season and climatic phenomena (Dey and Maiti 2013; Enshaeieh et al. 2013), or as a source of palmitoleic acid (Hernandez et al. 2013). While it is possible to choose the culture medium with a high C/N ratio (Chen et al. 2013; Papanikolaou and Aggelis 2011) to promote lipid accumulation, we chose a YPD medium with a non-limiting content of nutrients and C/N ratio of 3 to exclude other influences that might distort the impact of the use of lanthanides on growth, which has never been studied. It can therefore be assumed that further increase of lipid content would be supported by increasing the C/N ratio above 20, which is given as the minimum ratio leading to the increase of lipid accumulation (Dey and Maiti 2013; Fontanille et al. 2012; Papanikolaou and Aggelis 2011). According to the literature (Papanikolaou and Aggelis 2011) the lipid content of oleogenic yeast cells can only be enhanced by N or C limitations, yet we managed to increase the lipid content in oleogenic yeast by tens of percent by adding lanthanides to the culture. The maximum increase in oleaginous yeast was observed in *Y. lipolytica* from 17.9 to 25.1 % with lanthanum and in non-oleaginous yeast in *K. polysporus* from 5.1 to 9.1 %, again with lanthanum.

Low C/N ratio causes a low content of saturated fatty acids and increase in the content of unsaturated fatty acids (Braunwald et al. 2013; Mondala et al. 2012) and our results show that the addition of lanthanides causes a general decrease of saturated and mono-unsaturated FA and an increase of PUFA compared to the controls (a C/N ratio of 3).

The distribution of intracellular fatty acids is strain-dependent (Papanikolaou et al. 2009). In the case of yeast, the group of polyunsaturated fatty acids is represented only by linoleic acid (18:2). When the oleaginous and non-oleaginous yeasts were cultured with lanthanum, the increase in its content reached as much as 180 % compared to the control. Maximum increase was in *R. glutinis* from 13.1 to 36.8 % of total fatty acids and, among non-oleaginous yeasts, in *K. polysporus* from 2.7 to 7.7 % of total fatty acids.

The most interesting results were obtained in cultivations with monazite, a natural mineral that contains a

mixture of lanthanides in varying proportions dependent on monazite source. In terms of usefulness, addition of monazite (which is much cheaper than pure lanthanides) to yeast culture is highly beneficial, causing a high increase in cell mass, total lipids and nutritionally important fatty acids.

Cultivation of *K. polysporus* with monazite increased the polyunsaturated fatty acid content by up to 74 % compared with control. Other non-oleaginous yeasts increased the polyunsaturated fatty acid content in the presence of monazite by 50 and 39 % (*S. cerevisiae* and *T. delbrueckii*, respectively). The highest increase in oleaginous yeasts was 31 % in *R. glutinis*. Increasing the monazite concentration from 0.3 to 3 mg/l resulted in an increase of unsaturated fatty acid content in all seven strains. The increase was more than 10 % in non-oleaginous yeast and up to 10 %in oleaginous yeasts. Similar trends were obtained when culturing the cells under stressful conditions; thus the content of polyunsaturated fatty acids also increased in the presence of zero-valent iron nanoparticles (25 Nanofer) (Padrova et al. 2014) or under nitrogen limitation (Kolouchova et al. 2015b).

According to the literature the relative proportion of fatty acids is highly strain-dependent and a change of the culture conditions does not bring about any change of mutual ratios of fatty acids (Papanikolaou et al. 2004). However, nitrogen limitation or the presence of Nanofer25 can increase the proportion of nutritionally important fatty acids (Kolouchova et al. 2015b; Padrova et al. 2015). Non-oleaginous strains do not seem appropriate for producing palmitoleic and linoleic acid under our culture conditions. They have a very low content of lipids in dry matter (Fig. 1) and are not capable of increased lipid accumulation in the cytosol (Ageitos et al. 2011) unless their ability to accumulate lipid is increased by genetic manipulation (Yu et al. 2011). Although these strains are widely technologically used (Rezanka et al. 2013) and although the addition of lanthanides increased the proportion of palmitoleic and linoleic acid by about 50 % relative to the control and the yield by more than 100 % compared to the control, the resulting values do not reach the limits making them suitable for technological use. The highest yield of palmitoleic and linoleic acid, more than 300 mg/g, was achieved by culturing *T. cutaneum* either with lanthanum or with monazite (irrespective of concentration). Lanthanum or monazite increased the proportion of palmitoleic and linoleic acid and their yield by ten to twenty percent. *T. cutaneum* contains low amounts of palmitoleic acid (only about 3 %) but a high amount of linoleic acid (about 60 % of total fatty acids) comparable to that of cow milk, in which linoleic acid is also the major FA (Dhiman et al. 2000).

In order to try to decipher the possible mechanism of action of lanthanides, it may prove useful to abandon the generally adopted approach in which only covalent radii of individual ions are compared. When comparing. e.g., the covalent radii of La^{3+} and Ca^{2+} (2.07 vs. 1.76 Å) (Cordero et al. 2008), one of course compares the incomparable. However, the situation is completely different if we consider that both cations are present both outside and inside the cell in aqueous solution. Calcium forms complexes with water, with a coordination number of up to 10 (and it does not have valence f electrons!) of the type Ca $[H_2O]_8^{2+}$ or even Ca $[H_2O]_9^{2+}$) (Kaufman Katz et al. 1996) and, according to the coordination number of 8–10, its effective ionic radius is from 1.12 to 1.23 Å, whereas La^{3+}, which has a coordination number 8, has effective ionic radius 1.16 (Shannon 1976). This indicates the possibility of interaction between the cell and the lanthanides via the binding of complexes of trivalent lanthanides to protein binding sites instead of calcium. Calcium as a biogenic element is essential for the cells. Like calcium ions, lanthanide ions present in the culture may stabilize the structure of the cell wall and its ion exchange properties, control the activities of wall enzymes, etc. All of these physiological effects may lead to a change in the composition of lipids (fatty acids) in the cell walls, and also to a change in the composition of the triacylglycerols as storage substances. The presence of Ca^{2+} ions is also essential to the activity of phospholipases (Tang et al. 2002), which may thus be also affected by lanthanides. Another study has pointed out that La^{3+} and Gd^{3+} stabilize the hexagonal II phase in phosphatidylethanolamine (PE) membranes, that the chain-melting transition temperature of PE increases with increasing La^{3+} concentration and that, similar to Ca^{2+}, La^{3+} stabilizes the HII phase before the Lalpha phase (Tanaka et al. 2001). It has further been found that epidermal Ca^{2+} selective cation channel complex is influenced not only by calcium but also by other ions at low concentrations, e.g. by 0.5–2.0 μM Gd^{3+} and by somewhat higher concentrations of La^{3+} (Ding and Pickard 1993).

Conclusion

Lanthanum and monazite were found to influence most strongly the lipid content, cell mass and nutritionally important fatty acids in all seven oleaginous and non-oleaginous yeasts. The highest rise in lipid content (tens of percent compared to control) occurred on adding praseodymium or lanthanum to the cultures (22 % with *Candida sp.*, 40 % with *Y. lipolytica*, nearly 80 % with *K. polysporus*, 17 % with *S. cerevisiae* and 53 % with *T. delbrueckii*). Addition of 3 mg/l monazite increased the lipid content up to 50 % relative to control, especially in non-

oleaginous strains. The highest increase of the palmitoleic and linoleic acid content was brought about by lanthanum, and in some strains by monazite irrespective of its concentration. The highest yield of palmitoleic and linoleic acid, in excess of 300 mg/g, was obtained by culturing *T. cutaneum* with lanthanum and monazite. Lanthanides may probably act in the cell as substitutes for calcium ions and may give rise to both inducement and inhibition of the relevant enzymes whose activity is dependent on Ca^{2+}. These assumptions are so far only hypothetical but they deserve closer examination.

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