FEMS Yeast Research, 15, 2015, fov076

doi: 10.1093/femsyr/fov076
Advance Access Publication Date: 31 August 2015
Research Article

RESEARCH ARTICLE

Biotransformation of volatile fatty acids by oleaginous and non-oleaginous yeast species

Irena Kolouchová[1], Olga Schreiberová[1,*], Karel Sigler[2], Jan Masák[1] and Tomáš Řezanka[1,2]

[1]Department of Biotechnology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic and [2]Institute of Microbiology, CAS, Vídeňská 1083, 142 20 Prague, Czech Republic

*Corresponding author: Department of Biotechnology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic. Tel: +420-220-444-367; Fax: +420-224-311-082; E-mail: olga.schreiberova@vscht.cz

One sentence summary: This study points at the possible applications of oleaginous and non-oleaginous yeasts for biovalorization of industrial wastes by utilizing volatile fatty acids.

Editor: Hyun Ah Kang

ABSTRACT

The possibility of utilizing volatile fatty acids (VFA)-containing waste substrates from biotechnological and industrial processes was investigated by cultivating both oleaginous (*Candida* sp., *Rhodotorula glutinis*, *Trichosporon cutaneum*, *Yarrowia lipolytica*) and non-oleaginous (*Kluyveromyces polysporus*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*) yeast species on acetic acid, propionic acid and a combination of either acid with glucose as carbon and energy sources. Both oleaginous and non-oleaginous yeasts grew on VFA. Oleaginous yeasts accumulated lipids to 15–48% of dry cell weight, non-oleaginous yeasts also grew on VFA and showed comparable biomass yields but the lipid content was only 2–5%. Biomass and lipid yield increased in cultivations on VFA plus glucose. The lipid composition was comparable to plant-derived oils and therefore might be exploitable in biodiesel production; nearly all species, when cultured on propionate, showed a high content of the desirable odd-chain unsaturated FA, especially 17:1 acid. This study points at the wide array of possible applications of many yeasts, even non-oleaginous strains, for biovalorization of industrial wastes. Despite their low lipid content these species are useful because they can readily utilize VFA from waste products and, since they are not biologically hazardous, their biomass can be afterwards used, e.g. as livestock fodder.

Keywords: oleaginous yeasts; non-oleaginous yeasts; volatile fatty acids; biodiesel; microbial lipids; fatty acids

INTRODUCTION

Contemporary science is more and more frequently focusing on the possibilities of waste utilization, both from industry and other human activities. The goal is not only to lower the ecological impact and the amount of waste but also to meaningfully valorize residual substrates, e.g. for lipid accumulation by microorganisms (Aggelis *et al.* 1996; Fakas *et al.* 2006; Beopoulos *et al.* 2009).

Microorganisms have a broad array of metabolic activities that could be exploited in utilization of non-traditional substrates. Among these substrates belong VFA—volatile fatty acids (e.g. acetic acid and propionic acid) (du Preez *et al.* 1995, 1997; Chang *et al.* 2010; Fei *et al.* 2011b). Acetate is a by-product of biomass processing by the so-called anaerobic dark process for biohydrogen production. Acetic acid is frequently employed in food industry as an additive, in photography and in plastics manufacture; it is present in waste waters from butanol fermentation. Acetic acid is also generated by the hydrolysis of the acetyl groups present in hemicelluloses as part of processing of substrates in wood industry (Parajo, Dominguez and Dominguez

Received: 29 May 2015; **Accepted:** 2 August 2015

1998). VFA are by-products of anaerobic fermentation of organic residues (Lay, Lee and Noike 1999). There are many comprehensive studies about transformation of saccharides (e.g. glucose, fructose, lactose, sucrose, whey and xylose) to lipids, but the information available on the topic of lipid accumulation as a result of organic acid utilization is still insufficient (Fei *et al.* 2011b; Christophe *et al.* 2012a; Fontanille *et al.* 2012).

Many yeasts strains might be successfully exploited for cultivation on waste substrates. Some yeast species unable to accumulate lipids in high amounts, but with high biomass yields are usually described as non-oleaginous. Among these belong many biotechnological strains whose biomass can be used as livestock fodder. Non-oleaginous microorganisms accumulate less than 20% of lipids in dry cell weight (DCW) and synthesize polysaccharides such as glycogen, glucans and mannans as storage molecules (Donot *et al.* 2012).

Different metabolism characterizes oleaginous yeasts, which are able to convert certain organic acids directly to acetyl-CoA—the central intermediate of lipid biosynthesis—by acetyl-coenzyme A synthetase. Acetyl-CoA is subsequently utilized in fatty acid (FA) synthesis and results in lipid accumulation (Ratledge 2004; Papanikolaou and Aggelis 2011a, b). Oleaginous yeasts are studied for their ability to produce at least 20% of lipids per DCW (Papanikolaou and Aggelis 2011a). Among oleaginous species belong yeast genera *Cryptococcus*, *Trichosporon*, *Yarrowia*, *Candida* and *Rhodosporidium* sp. (Beopoulos *et al.* 2009; Ageitos *et al.* 2011; Papanikolaou and Aggelis 2011a, b). At the end of exponential phase and in the presence of metabolic stress lipid-accumulating microorganisms produce triacylglycerols (Meng *et al.* 2009), which are accumulated as lipid droplets in cytosol. Under the conditions of nitrogen or phosphorus limitation, the accumulation of lipids can reach 70% of DCW (Certik, Megova and Horenitzky 1999). Cultivations of oleaginous yeasts on hydrophobic substrates are often carried out with the addition of a hydrophilic carbon source such as glucose, but they can be cultivated on hydrophobic substrates as the only carbon and energy source (Donot *et al.* 2014).

One of the reasons behind the efforts to utilize VFA for lipid accumulation is to rationalize the economic balance of biodiesel production. Biodiesel is a mixture of fatty acid methyl esters (FAMEs), which can in future provide a potential alternative fuel for diesel engines and heating systems (Papanikolaou *et al.* 2008; Sharma and Singh 2009). The putative process of biodiesel production from microbial lipids is attractive for its low toxicity, possibility of by-product biodegradation and easy scale-up. At present the price of the microbial-derived oil is higher than that of the oil from fossil fuel sources, but if the efforts aimed at the process optimization are successful, i.e. including utilization of waste substrates coupled with high lipid/DCW yield or genetic engineering, the production might be competitive. The price of glucose constitutes 80% of the expenses of microbial lipids production with glucose as the only substrate (Fei *et al.* 2011a).

The ability of microorganisms to synthetize lipids has been suggested to be exploited also for production of lipids with unusual composition or as a substitute of some expensive products such as cocoa butter. Smit, Ykema and Verbree (1992) proposed a process utilizing waste by-products as substrates under oxygen-limiting conditions that lead to high stearate content in *Cryptococcus curvatus,* and although it was not found economical it showed that tailor-made lipid production could be performed with yeasts as biological agent.

Our work is focused on the evaluation of lipid production with VFA (acetic and propionic acid) as the only carbon and energy source and on a combination of VFA with glucose. For this study, we have chosen seven yeast species, representatives of four oleaginous and three technologically important non-oleaginous yeasts. The FA content and the proportion of positional isomers of heptadecenoic acid are monitored in different cultivation conditions, and the possibility of employing oleaginous and non-oleaginous yeast strains utilizing VFA for biotechnological processes is discussed.

MATERIALS AND METHODS

Microorganisms

The yeast strains used in the present study were *Candida* sp. DBM 2163, *Kluyveromyces polysporus* DBM 2171 (CCY 30-5-10), *Rhodotorula glutinis* CCY 20-2-20, *Saccharomyces cerevisiae* DBM 2115, *Torulaspora delbrueckii* DBM 39, *Trichosporon cutaneum* CCY 30-5-10 and *Yarrowia lipolytica* CCY 29-26-36 supplied by Culture Collection of Yeast (CCY), Institute of Chemistry, Slovak Academy of Science, Bratislava and by Collection of Yeasts and Industrial Microorganisms (DBM) of University of Chemistry and Technology, Prague. For long-term storage, the stock cultures were maintained in 20% glycerol at –60 °C.

Cultivation conditions

The precultures of yeast strains were cultivated in 200 ml of YPD medium (20 g l^{-1} peptone, 10 g l^{-1} yeast extract, 20 g l^{-1} glucose, initial pH 6.0) in Erlenmeyer flasks on a rotary shaker at 150 rpm at 28°C to the late exponential growth phase (26 h).

For lipid production, 200 ml of mineral medium in 500 ml Erlenmeyer flasks was inoculated with 10 ml of preculture to a final concentration of OD_{600} 0.2 and incubated on a rotary shaker at 150 rpm and 28°C. The biomass for analysis was harvested by centrifugation (9000 g, 10 min) in the stationary phase of growth, until the assimilation of all substrates according to Fontanille *et al.* (2012) and Peng *et al.* (2013).

The mineral medium composition was (g per liter): KH_2PO_4 (3.5), Na_2HPO_4 (2), $MgSO_4.7\ H_2O$ (1.5), NH_4Cl (1.5), yeast extract (1.5) and 1 ml of a trace element solution. The trace element solution contained (g per liter) $MnCl_2 \cdot 4H_2O$ (20), $CaCl_2 \cdot 2H_2O$ (20), $FeSO_4 \cdot 7H_2O$ (1) and $NaMoO_4 \cdot 2H_2O$ (1). The pH was adjusted to 6.0 VFA (acetic acid 4 g l^{-1} or propionic acid 4 g l^{-1}); VFA and glucose (acetic acid 4 g l^{-1} and glucose 20 g l^{-1} or propionic acid 4 g l^{-1} and glucose 20 g l^{-1}) were added as a carbon source. Cultivations with glucose (20 g l^{-1}) as the sole carbon source were used as control. All experiments were performed at least in triplicate.

Determination of DCW

For the DCW determination, 10 ml of medium in stationary phase of growth was filtered using pre-dried and weighed nitrocellulose filter (0.45 μm, Millipore) through vacuum pump, and the sample was washed several times. The DCW was determined after drying the samples to a constant weight at 110°C.

Lipid extraction

After harvesting, the yeast biomass was lyophilized and subsequently mixed with 2 ml of 0.1 M Na_2CO_3. The mixture was repeatedly ground with ballotini glass beads (diameter 0.2 mm) in a mortar, overlaid with liquid nitrogen. After three cycles, final volume 50 ml of 0.1 M Na_2CO_3 was added. The resulting crushed biomass 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.

FAMEs analysis

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

Gas chromatography–mass spectrometry of FAME was performed 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) was used for separation (100 m, 0.25 mm ID, 0.200-μm film thickness). 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 were identified according to their mass spectra (Vancura *et al.* 1987) and using a mixture of chemical standards obtained from Sigma-Aldrich.

Statistical analysis

The program CANOCO 5 (Microcomputer Power, Ithaca, NY, USA) was used to perform multivariate statistical analyses, Principal component analysis (PCA) was employed to visualize the variability within the dataset.

RESULTS AND DISCUSSION

Cultivation on volatile FAs as sole carbon source

There is only a limited amount of published data on the cultivation on VFA as the sole carbon source. The available publications concentrated on a few microorganisms (mostly *C. curvatus* and *Y. lipolytica*); we therefore carried out a screening of seven yeast strains, studying cultivations on acetic and propionic acids, both separately and in mixture with glucose.

The initial concentration 4 g l^{-1} of VFA was chosen based on the results published by Fei *et al.* (2011b), in which the highest lipid yield was obtained on VFA concentration 2 g l^{-1}, but the concentration 5 g l^{-1} lead only to 2% decrease from 27 to 25%. The concentration 5 g l^{-1} is usually considered as the most suitable for lipid accumulation by *C. albidus* and *Rhodosporidium toruloides* (Rodrigues and Pais 2000; Yahara *et al.* 2007; Papanikolaou and Aggelis 2011b; Zhao *et al.* 2012).

Table 1 lists the biomass yield (X, g l^{-1}), total lipid amount produced in cells (% w/w) and the amount of lipids (L, g l^{-1}) of the seven studied yeast strains. The cultivations were carried out on acetic acid (4 g l^{-1}), propionic acid (4 g l^{-1}) and a mixture of glucose (20 g l^{-1}) with acetic acid (4 g l^{-1}) or glucose (20 g l^{-1}) with propionic acid (4 g l^{-1}). The data show that cells were able to grow to higher cell densities on acetic acid in comparison with propionic acid. The biomass yield on acetic acid reached 25% in *Candida* sp. but up to 160 relative% (glucose with acetic acid) and 100% (glucose with propionic acid) in the non-oleaginous yeast *S. cerevisiae*. Acetic acid was assimilated more effectively for lipid accumulation than propionic acid, as was already observed by Fontanille *et al.* (2012) and du Preez, Immelman and Kilian (1996).

However, some studies attest to different trends—both inhibitory, e.g. Fontanille *et al.* (2012) who reported inhibition of *Y. lipolytica* growth on initial VFA concentration 5 g l^{-1}, and positive—Lian *et al.* (2012) described the highest *C. curvatus* biomass yields on 30 g l^{-1} of acetate and inhibition was not observed until the concentration of 60 g l^{-1}. Cultivations in the presence of excess glucose were used as a control. Our results show that all the selected strains were able to grow both on acetic and propionic acid, albeit with low growth rates. The growth rate on acetic acid was between 0.02 and 0.04 h^{-1} for all strains and cultivation on propionic acid led to similar growth rates, 0.02–0.04 h^{-1}. In comparison, the growth rate on glucose was 0.11 h^{-1} (*T. cutaneum*) and 0.19/h (*S. cerevisiae*). In the case of cultivation on glucose and acetic acid, the growth rates were 0.03–0.05 h^{-1}. Glucose and propionic acid mixture as substrate led to growth rates 0.02–0.04 h^{-1}. It is apparent that addition of glucose did not diminish the inhibitory effects of VFA on growth rate. Our results show lower biomass yield than the results reported by Christophe *et al.* (2012a) and others (Papanikolaou and Aggelis 2011a, b). This can be explained by the use of different strains than reported in the literature, by our selection of species that had not been extensively studied with regard to utilization of VFA and therefore there are not available comparative studies, and also by a different experimental set-up. Another important factor is that the batch cultivation, which was chosen for our screening experiments, provides lower biomass yields than fed-batch or continuous cultivation (Wynn, bin Abdul Hamid and Ratledge 1999; Beopoulos *et al.* 2009; Papanikolaou and Aggelis 2011a).

Effect of acetic acid and propionic acid on lipid content

De novo lipid synthesis and *ex novo* lipid accumulation are two main pathways of lipid synthesis by cells (Papanikolaou and Aggelis 2011b). *De novo* lipid accumulation is the result of cultivation on hydrophilic substrates—saccharides or short-chain FAs (e.g. in waste water utilization) (Peng *et al.* 2013). Cultivation of microorganisms on hydrophobic substrates as the only carbon source leads to *ex novo* lipid accumulation. The accumulation of *ex novo* lipids is a primary anabolic process occurring simultaneously with the production of lipid-free material (Fei *et al.* 2011b; Donot *et al.* 2014).

During cultivation on different substrates, the lipid content of cells is gradually changing. These changes are dependent on substrate type, duration of cultivation as well as inoculum size, temperature, pH and other factors (Fakas *et al.* 2006, 2007; Papanikolaou and Aggelis 2011b; Peng *et al.* 2013). We used the cultivation temperature of 28°C according to the data by Fei *et al.* (2011b) and initial medium pH 6. Under these conditions, the maximum lipid accumulation was observed. Cultivation at standard temperature also improves the economic balance of the process on the commercial scale. Beside temperature, pH, inoculum size and carbon source, the lipid accumulation is highly dependent on nitrogen source. In cultivation on VFA, Fei *et al.* (2011b) tested several different nitrogen sources and NH_4Cl was found to best support lipid accumulation. Accordingly, we chose NH_4Cl as nitrogen source in our experiments. The lipid content was determined in stationary phase of growth—96 h for cultivation on VFA according to Peng *et al.* (2013), 26 h for the control cultivation on glucose. As shown in Table 1, the highest lipid yield of 15–43% was observed in cultivations of *Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica* on VFA on a combination of glucose and VFA. More than 40% of lipid content was observed only in *T. cutaneum* cultivation. Similar values were

Table 1. Effect of utilization of different substrates (glucose G; acetic acid A; propionic acid P) on biomass yield (X), total lipid production (L) and lipid yield by oleaginous (*Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica*) and non-oleaginous (*K. polysporus*, *S. cerevisiae* and *To. delbrueckii*) yeast species. Cultivation was performed until stationary phase.

Strain	Source[a]	X[b] (g l^{-1})	r[c] (g l^{-1})	L[d] (g l^{-1})	Lipids (% w/w)
Candida sp.	G	4.46	0.17	1.58	35.40
	A	3.15	0.03	0.76	24.20
	G + A	3.95	0.04	1.31	33.20
	P	1.98	0.02	0.31	15.70
	G + P	2.44	0.03	0.64	26.40
Rhodotorula glutinis	G	3.25	0.13	1.08	33.20
	A	2.84	0.03	0.55	19.50
	G + A	3.17	0.03	0.90	28.40
	P	1.52	0.02	0.22	14.60
	G + P	1.95	0.02	0.37	18.90
Trichosporon cutaneum	G	2.93	0.11	1.43	48.90
	A	2.64	0.03	0.88	33.20
	G + A	2.72	0.03	1.16	42.80
	P	1.60	0.02	0.38	23.90
	G + P	1.76	0.02	0.64	36.10
Yarrowia lipolytica	G	4.40	0.17	0.79	17.90
	A	4.21	0.04	0.56	13.30
	G + A	4.31	0.04	0.71	16.50
	P	3.53	0.04	0.31	8.90
	G + P	3.83	0.04	0.39	10.20
Kluyveromyces polysporus	G	4.51	0.17	0.23	5.10
	A	4.02	0.04	0.13	3.20
	G + A	4.22	0.04	0.20	4.80
	P	3.24	0.03	0.07	2.10
	G + P	3.56	0.04	0.13	3.60
Saccharomyces cerevisiae	G	4.96	0.19	0.19	3.80
	A	1.66	0.02	0.05	2.80
	G + A	4.43	0.05	0.16	3.70
	P	1.59	0.02	0.04	2.40
	G + P	3.19	0.03	0.10	3.10
Torulaspora delbrueckii	G	4.46	0.17	0.21	4.60
	A	3.69	0.04	0.14	3.80
	G + A	4.04	0.04	0.17	4.10
	P	2.82	0.04	0.08	2.70
	G + P	3.00	0.04	0.09	3.00

a) Carbon source G-glucose, A - acetic acid, P - propionic acid.
b) X is the DCW produced (in g l^{-1}).
c) r is the growth rate (in h^{-1}).
d) L is the total cellular lipid produced (in g l^{-1}).

reported by Fontanille *et al.* (2012), who cultivated *Y. lipolytica* in a fed-batch process and obtained higher biomass yields and lipid content in g l^{-1}. In his study, an increase of working volume led to equivalent increase of these values, but only in cultivation on VFA/glucose mixture. The reported biomass and lipid yields of fed-batch cultivation on VFA alone were comparable to our results—non-oleaginous yeasts *S. cerevisiae*, *To. delbrueckii* and *K. polysporus* were also able to grow on non-traditional substrates and the biomass yields were comparable to oleaginous strains but, predictably, the lipid content was only 2–5%. If fed-batch cultivation or scale-up was performed and higher biomass yield would be achieved (Rezanka *et al.* 2013b), it is probable that the obtained biomass could be utilized as livestock fodder or in dietary supplements.

FA composition of lipids

The influence of substrate on lipid composition of the seven yeast strains was determined by GC-MS. Table 2 shows the ratio of saturated and unsaturated FAs. The lowest unsaturated FAs percentage, 70%, was observed in *To. delbrueckii*. The highest proportion of unsaturated FAs was found in *K. polysporus*, reaching up to 90%.

This work is the first instance of comparison of several species of both oleaginous and non-oleaginous yeast cultivated on unusual substrates. Both oleaginous and non-oleaginous strains can be exploited in biodiesel production, as (without regard to lipid accumulation) they produced the required high content of polyunsaturated FAs. The percentage of unsaturated FAs in one of the most studied oleaginous yeasts *Y. lipolytica* was around 80%, which is in accordance with results published by Fontanille *et al.* (2012). This high percentage of unsaturated FAs is partially induced by ammonium ions as nitrogen source. In a study on *C. curvatus* (Zheng *et al.* 2012) using also ammonium as nitrogen source, the content of linoleic acid (18:2) increased in comparison with stearic acid (18:0), but the ratio of these FAs was not reversed.

As demonstrated in Figs 1 and 2, the highest lipid content was found in cultivations on glucose, the same trend having been observed by Papanikolaou and Aggelis (2011a, b).

Table 2. Proportion of saturated and unsaturated FAs in the oleaginous (*Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica*) and non-oleaginous (*K. polysporus*, *S. cerevisiae* and *To. delbrueckii*) yeast strains cultivated on different substrates (glucose G; acetic acid A; propionic acid P).

Strain	Source[a]	saturated FA	unsaturated FA
Candida sp.	G	22.9	77.1
	A	22.1	77.9
	G + A	12.5	87.5
	P	22.1	77.9
	G + P	15.5	84.5
Rhodotorula glutinis	G	21.6	78.4
	A	21.9	78.1
	G + A	19.5	80.5
	P	20.8	79.2
	G + P	20.9	79.1
Trichosporon cutaneum	G	22.5	77.5
	A	21.0	79.0
	G + A	19.1	80.9
	P	22.6	77.4
	G + P	20.1	79.9
Yarrowia lipolytica	G	20.4	79.6
	A	19.6	80.4
	G + A	15.7	84.3
	P	19.4	80.6
	G + P	17.6	82.4
Kluyveromyces polysporus	G	9.3	90.7
	A	7.2	92.8
	G + A	4.7	95.3
	P	9.1	90.9
	G + P	8.0	92.0
Saccharomyces cerevisiae	G	19.3	80.7
	A	19.3	80.7
	G + A	16.8	83.2
	P	19.5	80.5
	G + P	19.0	81.0
Torulaspora delbrueckii	G	35.9	64.1
	A	29.7	70.3
	G + A	21.8	78.2
	P	32.2	67.8
	G + P	27.8	72.2

a) Carbon source G—glucose, A—acetic acid, P—propionic acid

Comparable results were obtained in cultivations on glucose–acetic acid mixture. The most common FAs in cultivations of *C. albidus*, *T. dermatis* and *Rh. toruloides* are palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2), respectively (Ratledge and Wynn 2002; Papanikolaou *et al.* 2009; Fei *et al.* 2011b; Fontanille *et al.* 2012; Zhao *et al.* 2012; Peng *et al.* 2013). The same FAs were found in the present study in the oleaginous yeasts *Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica* cultured on any of the substrates. This FA composition is similar to oils that are used as substrates for biofuel production in the United States and the European Union (Liang *et al.* 2010; Christophe *et al.* 2012b; Peng *et al.* 2013). The higher the content of unsaturated FAs the better is their applicability in biodiesel production. When considering the FA composition, our data suggest that *Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica* are suitable for VFA-based biodiesel production. PCA (Fig. 3) confirmed these data, separating the cultivations on widely different carbon sources along the first canonical axis. This data analysis shows the suitability of a specific microorganism and substrate for a defined biotechnological production of a given FA. The more is a specific strain cultivated on a specific substrate removed from the center the more suitable it is for specific FA production. For example, as apparent from Fig. 3, linoleic acid (18:2) can be produced by *T. cutaneum*. If these yeasts were cultivated in fed-batch mode (Fontanille *et al.* 2012) and with the employment of the simulation models of microbial lipid production as described by Park *et al.* (2014), they might be a promising source of microbial lipids for technological purposes.

Influence of VFA on the content of odd-chain FAs

The content of odd-chain FAs was influenced most strongly by propionic acid as substrate. The presence of propionic acid as substrate led to an increase of heptadecenoic acid (17:1) in all studied strains (Figs 1 and 2), as also reported in other studies (Zheng *et al.* 2012; Rezanka *et al.* 2013a). Its increase in *Candida* sp., *K. polysporus*, *To. delbrueckii* and *T. cutaneum* was up to 37% in comparison with *S. cerevisiae* (5.1%) or *R. glutinis* (18.8%). The reason for the high content of odd-chain FAs might be the presence of a special metabolic pathway of propionate that could directly react with CoA to form propionyl-COA, a precursor in the synthesis of odd-numbered FAs. In the presence of other substrates, the content of heptadecenoic acid in all studied strains was 0–3.8%. Different substrates also influenced the proportional

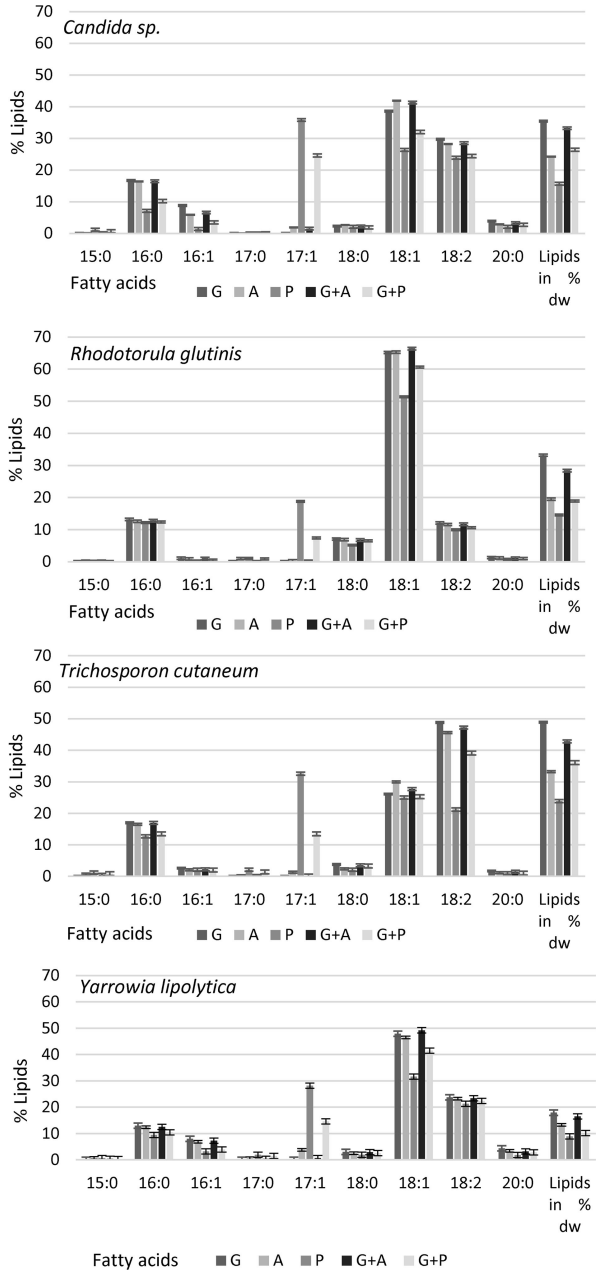

Figure 1. Lipid composition of *Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica* during cultivation on different carbon sources (glucose G; acetic acid A; propionic acid P).

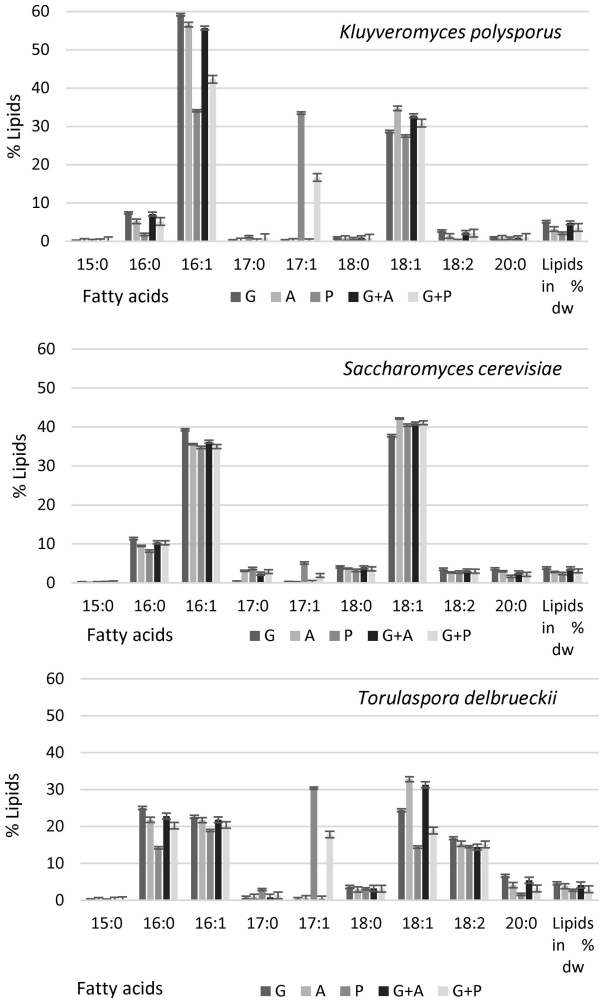

Figure 2. Lipid composition of *K. polysporus*, *S. cerevisiae* and *To. delbrueckii* during cultivation on different carbon sources (glucose G; acetic acid A; propionic acid P).

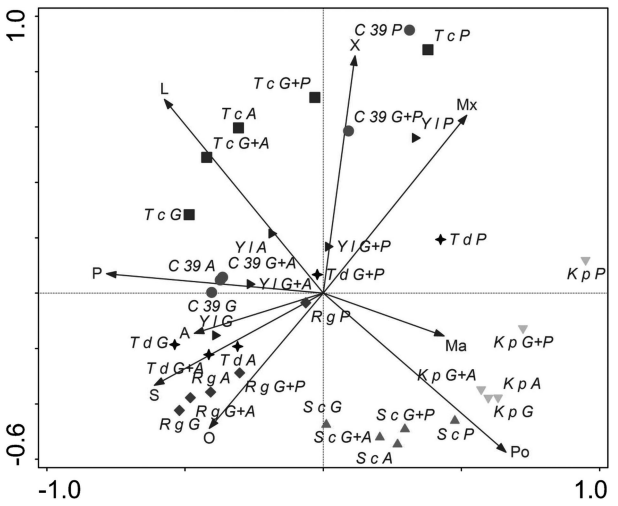

Figure 3. PCA of FA composition in the seven yeast species (*Candida* sp. C; *Kluyveromyces polysporus K p*; *Rhodotorula glutinis R g*; *Saccharomyces cerevisiae S c*; *Torulaspora delbrueckii T d*; *Trichosporon cutaneum T c*; *Yarrowia lipolytica Y l*) cultivated with different carbon sources (glucose G; acetic acid A; propionic acid P). Fatty acids: 15:0 (X), 16:0 (P), 16:1 (Po), 17:0 (Ma), 17:1 (Mx), 18:0 (S), 18:1 (O), 18:2 (L), 20:0 (A).

content of positional isomers of heptadecenoic acid (Fig. 4). Even more significant changes were observed in the content of palmitoleic acid (16:1), 20–60 relative% in cultivation on all studied substrates in *K. polysporus*, *S. cerevisiae* and *To. delbrueckii*. The content of oleic acid (18:1) was also high (15–66% in *R. glutinis*) in most of the cultivation conditions and yeast strains and linoleic acid (18:2) was up to 50% in *T. cutaneum* biomass and, in contrast, only 0.2–3.5% in *K. polysporus* and *S. cerevisiae*.

CONCLUSIONS

Lipid accumulation was studied in VFA cultivations of seven oleaginous and non-oleaginous yeast strains. High content of

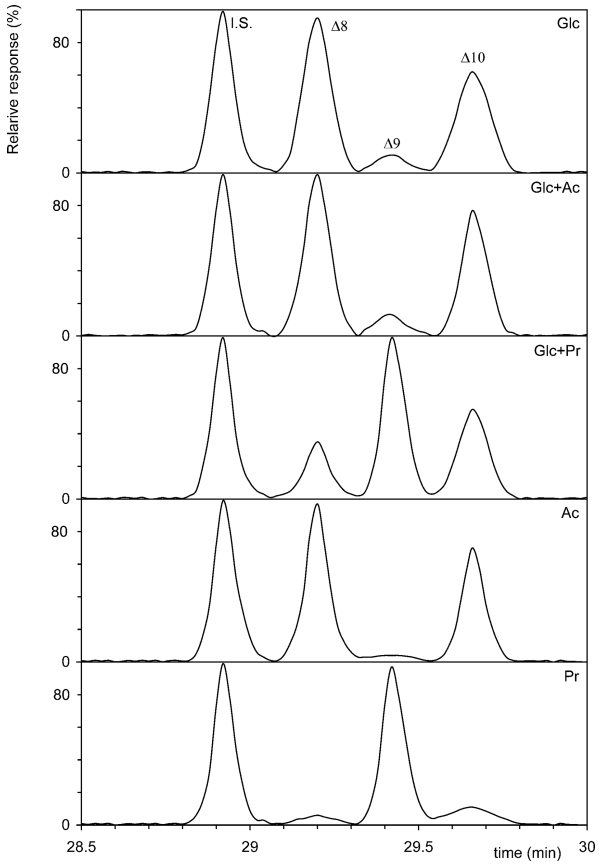

Figure 4. Chromatogram of positional isomers of heptadecenoic acid derivatives analyzed by GC-MS (electron impact ionization).

unsaturated FAs in cell lipids was found in all the studied yeast strains. The highest lipid to biomass ratio was observed in *Candida* sp., *R. glutinis*, *T. cutaneum* and *Y. lipolytica*. These oleaginous yeasts might be employed for bioconversion of VFA into biofuels, help with the remediation of environment and simultaneously utilize cheap substrates for biodiesel production. Although non-oleaginous do not reach the high lipid production that is observed in the oleaginous strains, they are able to utilize waste substrates as carbon and energy sources. They can be therefore used in biotechnological processes other than biodiesel production, for dietary supplements or as livestock fodder due to their advantages such as microbiological safety and easy biomass acquisition. However, before potential scale-up and commercialization, the process must be further investigated and optimized.

FUNDING

This work was supported by the Grant Agency of the Czech Republic [P 503 14–00227S].

Conflict of interest. None declared.

REFERENCES

Ageitos JM, Vallejo JA, Veiga-Crespo P, *et al.* Oily yeasts as oleaginous cell factories. *Appl Microbiol Biot* 2011;**90**:1219–27.

Aggelis G, Stathas D, Tavoularis N, *et al.* Composition of lipids produced by some strains of *Candida* species. Production of single-cell oil in a chemostat culture. *Folia Microbiol* 1996;**41**:299–302.

Beopoulos A, Cescut J, Haddouche R, *et al. Yarrowia lipolytica* as a model for bio-oil production. *Prog Lipid Res* 2009;**48**:375–87.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 1959;**37**:911–7.

Certik M, Megova J, Horenitzky R. Effect of nitrogen sources on the activities of lipogenic enzymes in oleaginous fungus *Cunninghamella echinulata*. *J Gen Appl Microbiol* 1999;**45**:289–93.

Chang HN, Kim NJ, Kang J, *et al.* Biomass-derived volatile fatty acid platform for fuels and chemicals. *Biotechnol Bioproc E* 2010;**15**:1–10.

Christophe G, Deo JL, Kumar V, *et al.* Production of oils from acetic acid by the oleaginous yeast *Cryptococcus curvatus*. *Appl Biochem Biotech* 2012a;**167**:1270–9.

Christophe G, Kumar V, Nouaille R, *et al.* Recent developments in microbial oils production: a possible alternative to vegetable oils for biodiesel without competition with human food? *Braz Arch Biol Techn* 2012b;**55**:29–46.

Donot F, Fontana A, Baccou JC, *et al.* Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. *Carbohyd Polym* 2012;**87**:951–62.

Donot F, Fontana A, Baccou JC, *et al.* Single cell oils (SCOs) from oleaginous yeasts and moulds: production and genetics. *Biomass Bioenerg* 2014;**68**:135–50.

du Preez JC, Immelman M, Kilian SG. The utilization of short-chain monocarboxylic acids as carbon sources for the production of gamma-linolenic acid by *Mucor* strains in fed-batch culture. *World J Microb Biot* 1996;**12**:68–72.

du Preez JC, Immelman M, Kock JLF, *et al.* Production of gamma-linolenic acid by *Mucor circinelloides* and *Mucor rouxii* with acetic acid as carbon substrate. *Biotechnol Lett* 1995;**17**:933–8.

du Preez JC, Immelman M, Kock JLF, *et al.* The effect of acetic acid concentration on the growth and production of gamma-linolenic acid by *Mucor circinelloides* CBS 203.28 in fed-batch culture. *World J Microb Biot* 1997;**13**:81–7.

Fakas S, Galiotou-Panayotou M, Papanikolaou S, *et al.* Compositional shifts in lipid fractions during lipid turnover in *Cunninghamella echinulata*. *Enzyme Microb Tech* 2007;**40**:1321–7.

Fakas S, Papanikolaou S, Galiotou-Panayotou M, *et al.* Lipids of *Cunninghamella echinulata* with emphasis to gamma-linolenic acid distribution among lipid classes. *Appl Microbiol Biot* 2006;**73**:676–83.

Fei Q, Chang HN, Shang L, *et al.* Exploring low-cost carbon sources for microbial lipids production by fed-batch cultivation of *Cryptococcus albidus*. *Biotechnol Bioproc E* 2011a;**16**:482–7.

Fei Q, Chang HN, Shang LA, *et al.* The effect of volatile fatty acids as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production. *Bioresource Technol* 2011b;**102**:2695–701.

Fontanille P, Kumar V, Christophe G, *et al.* Bioconversion of volatile fatty acids into lipids by the oleaginous yeast *Yarrowia lipolytica*. *Bioresource Technol* 2012;**114**:443–9.

Lay JJ, Lee YJ, Noike T. Feasibility of biological hydrogen production from organic fraction of municipal solid waste. *Water Res* 1999;**33**:2579–86.

Lian J, Garcia-Perez M, Coates R, *et al.* Yeast fermentation of carboxylic acids obtained from pyrolytic aqueous phases for lipid production. *Bioresource Technol* 2012;**118**:177–86.

Liang YN, Cui Y, Trushenski J, *et al*. Converting crude glycerol derived from yellow grease to lipids through yeast fermentation. *Bioresource Technol* 2010;**101**:7581–6.

Meng X, Yang JM, Xu X, *et al*. Biodiesel production from oleaginous microorganisms. *Renew Energ* 2009;**34**:1–5.

Papanikolaou S, Aggelis G. Lipids of oleaginous yeasts. Part I: biochemistry of single cell oil production. *Eur J Lipid Sci Tech* 2011a;**113**:1031–51.

Papanikolaou S, Aggelis G. Lipids of oleaginous yeasts. Part II: Technology and potential applications. *Eur J Lipid Sci Tech* 2011b;**113**:1052–73.

Papanikolaou S, Chatzifragkou A, Fakas S, *et al*. Biosynthesis of lipids and organic acids by *Yarrowia lipolytica* strains cultivated on glucose. *Eur J Lipid Sci Tech* 2009;**111**:1221–32.

Papanikolaou S, Fakas S, Fick M, *et al*. Biotechnological valorisation of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: Production of 1,3-propanediol, citric acid and single cell oil. *Biomass Bioenerg* 2008;**32**:60–71.

Parajo JC, Dominguez H, Dominguez JM. Biotechnological production of xylitol. Part 3: operation in culture media made from lignocellulose hydrolysates. *Bioresource Technol* 1998;**66**:25–40.

Park GW, Fei Q, Jung K, *et al*. Volatile fatty acids derived from waste organics provide an economical carbon source for microbial lipids/biodiesel production. *Biotechnol J* 2014;**9**:1536–46.

Peng WF, Huang C, Chen XF, *et al*. Microbial conversion of wastewater from butanol fermentation to microbial oil by oleaginous yeast *Trichosporon derrnatis*. *Renew Energ* 2013;**55**:31–4.

Ratledge C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie* 2004;**86**:807–15.

Ratledge C, Wynn JP. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv Appl Microbiol* 2002;**51**:1–51.

Rezanka T, Kolouchova I, Cejkova A, *et al*. Identification of regioisomers and enantiomers of triacylglycerols in different yeasts using reversed- and chiral-phase LC-MS. *J Sep Sci* 2013a;**36**:3310–20.

Rezanka T, Matoulkova D, Kolouchova I, *et al*. Brewer's yeast as a new source of palmitoleic acid-analysis of triacylglycerols by LC-MS. *J Am Oil Chem Soc* 2013b;**90**:1327–42.

Rodrigues G, Pais C. The influence of acetic and other weak carboxylic acids on growth and cellular death of the yeast *Yarrowia lipolytica*. *Food Technol Biotech* 2000;**38**:27–32.

Sharma YC, Singh B. Development of biodiesel: current scenario. *Renew Sust Energ Rev* 2009;**13**:1646–51.

Smit H, Ykema A, Verbree EC 'Production of cocoa butter equivalents by yeast mutants.' In: *Industrial Applications of Single Cell Oils*. Kyle DC, Ratledge C, *et al*. (eds). Champaign, IL: AOCS Press, 1992;182–95.

Vancura A, Rezanka T, Marsalek J, *et al*. Effect of ammonium ions on the composition of fatty acids in *Streptomyces fradiae*, producer of tylosin. *FEMS Microbiol Lett* 1987;**48**:357–60.

Wynn JP, bin Abdul Hamid A, Ratledge C. The role of malic enzyme in the regulation of lipid accumulation in filamentous fungi. *Microbiology* 1999;**145**:1911–7.

Yahara GA, Javier MA, Tulio MJM, *et al*. Modeling of yeast *Brettanomyces bruxellensis* growth at different acetic acid concentrations under aerobic and anaerobic conditions. *Bioproc Biosyst Eng* 2007;**30**:389–95.

Zhao XB, Peng F, Du W, *et al*. Effects of some inhibitors on the growth and lipid accumulation of oleaginous yeast *Rhodosporidium toruloides* and preparation of biodiesel by enzymatic transesterification of the lipid. *Bioproc Biosyst Eng* 2012;**35**:993–1004.

Zheng Y, Chi Z, Ahring BK, *et al*. Oleaginous yeast *Cryptococcus curvatus* for biofuel production: ammonia's effect. *Biomass Bioenergy* 2012;**37**:114–21.