Folia Microbiol (2015) 60:457–464
DOI 10.1007/s12223-015-0388-9

Precursor directed biosynthesis of odd-numbered fatty acids by different yeasts

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Received: 10 December 2014 / Accepted: 15 March 2015 / Published online: 27 March 2015

Abstract Precursor-directed biosynthesis was used for directed preparation of positional isomers of heptadecanoic acid (17:1), which have convenient pharmacological properties. Cultivation of *Candida* sp., *Kluyveromyces polysporus, Rhodotorula glutinis, Saccharomyces cerevisiae, Torulaspora delbrueckii, Trichosporon cutaneum*, and *Yarrowia lipolytica* on 20 g/L glucose, 4 g/L acetic, or 4 g/L propionic acids yielded different proportions of 17:1. Cultivation on carbon sources with even numbers of carbon atoms (glucose and acetic acid) produced preferentially 8Z- and 10Z-heptadecenoic acids in about equal amounts, in agreement with the proposed biosynthesis of fatty acids, whereas cultivation on propionic acid as the only carbon source produced over 90 % of total fatty acids of 9-17:1 out of all possible positional isomers. The structures of positional isomers of 17:1 acid were determined using dimethyl disulfides of fatty acid methyl esters. In cultivation of *Candida* sp. on propionic acid, the yield of heptadecenoic acid reached 111 mg/L cultivation medium. Principal component analysis was used for identifying the effect of cultivation conditions on the production of the 17:1 acid by individual yeast strains.

Electronic supplementary material The online version of this article (doi:10.1007/s12223-015-0388-9) contains supplementary material, which is available to authorized users.

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Introduction

Heptadecenoic acid (17:1) is an odd-numbered fatty acid (FA) with a single double bond. It is quite widespread in nature from bacteria to higher green plants and mammals, but in all organisms, it is usually present as a minor fatty acid.

It has important pharmacological properties. It functions as an anti-inflammatory and edema-inhibiting compound, and it is also active on psoriasis, allergies, and autoimmune diseases, especially in prophylaxis (Degwert et al. 1998). Other biological effects of heptadecenoic acid were described by Avis et al. (2000); the compound was used as chloroform extracts from a culture broth of the fungus *Pseudozyma flocculosa* (syn. *Sporothrix flocculosa*) which is a yeast-like fungus with antagonistic activity against powdery mildew fungi. The fungus was found to contain two fatty acids with antibiotic activity, viz. 9-heptadecenoic and 6-methyl-9-heptadecenoic acids. Isolation, extraction, and purification of these fatty acids from culture filtrates is difficult and results in only low and unreliable yields. This led to the total synthesis of both acids (Benyagoub et al. 1996) which in turn resulted in a detailed description of their biological effects. The mechanism of inhibition of the powdery mildew by heptadecenoic acid was explained in terms of membrane disruption resulting in the release of intracellular electrolytes and proteins and eventually cytoplasmic disintegration of mycelia (Avis and Belanger 2001).

A key step in the synthesis of fatty acids is the production of a biosynthetic precursor, i.e., acyl-CoA. Odd chain fatty acids with straight chains are biosynthesized most often from odd-numbered chain precursors, i.e., propionyl-CoA (methylmalonyl-CoA), or arise by chain shortening of even chain FAs by α-oxidation. The yeast *Saccharomyces cerevisiae* has been described to be unable to grow on propionic acid as the sole carbon source; addition of the acid to a glucose-limited

chemostat culture leads to increased activity of propionyl-CoA synthetase that could facilitate the synthesis of heptadecenoic acid. However, propionyl-CoA is at the same time increasingly degraded to pyruvate and thus also acetate by the methylcitrate pathway (Pronk et al. 1994). By contrast, the yeast *Cryptococcus albidus* can grow on propionate and produce heptadecenoic acid (Fei et al. 2011).

Another possibility of synthesizing heptadecenoic acid is the directed biosynthesis of FAs making use of odd chain precursors. *Candida tropicalis* was cultivated on a mixture of hydrocarbons from C13 to C18 and heptadecenoic acid in an amount of 17 g (from 4 kg of yeast) that was 99 % pure and was isolated in three cycles and 21 crystallization steps (Bauchart and Aurousseau 1980).

When the yeast *Yarrowia lipolytica* (Fontanille et al. 2012) was cultivated on a mixture of glycerol and propionic acid, analysis of FAs revealed about 20 % of unidentified fatty acid methyl esters (FAMEs). Based on other data (Zhelifonova et al. 1974), which describe the growth of *C. tropicalis* on acetic, lactic, and propionic acids and analysis of FAs, we assume that the unidentified FAs are in fact odd-numbered FAs. Odd chain FAs, including unsaturated ones, were also prepared by using recombinant microbial cells; however, examples have been reported only for wild-type strains of *Escherichia coli* which already produce odd numbered FAs (Lee et al. 2013). The situation with the production and identification of 17:1 is additionally complicated by the existence of several positional isomers of heptadecenoic acid that occur in nature but have not yet been determined. More than one positional isomer has been described in taxonomically distinct sources.

Identification of positional isomers of heptadecenoic acid has been satisfactorily concluded only very recently. The Delmonte group (Delmonte et al. 2008; Santercole et al. 2012; Fardin-Kia et al. 2013) prepared and analyzed positional isomers of 17:1 by using 100-m capillary highly polar columns. They succeeded (Fardin-Kia et al. 2013) in separating almost all positional isomers of 17:1.

The sources most often studied in terms of occurrence of 17:1 acid are mammalian milk and fat (Alves et al. 2006), seeds of Malvaceae family plants (Dowd 2012; Dowd and Farve 2013), and higher basidiomycete fungi (Pedneault et al. 2008). Mammalian milk was found to contain cis-8 and cis-9-17:1, probably of rumen microbial origin. Heptadecenoic acid can also be produced by mammary glands. The seeds of Malvaceae plants (*Thespesia populnea*, *Gossypium hirsutum*, and *Tilia* spp.) were found to contain 8-17:1 as a major acid and 9- and 10-17:1 as minor ones (Dowd 2012; Dowd and Farve 2013). The fungus *Ganoderma applanatum* was found to contain cis-8-, cis-9- and cis-11-17:1 and the position of the double bond was determined based on mass spectra of picolinyl esters (Pedneault et al. 2008).

A number of methods have been developed for localization of double bonds in FAME chains (Christie and Han 2010). One of the frequently used methods is the derivatization of double bond in FAME by dimethyl disulfide adducts that "fixes" the double bond. These compounds are very easy to prepare and have excellent mass spectrometric properties. The mass spectrum displays four dominant types of ions, i.e. $[M]^+$ ion, two ions (aliphatic and carboxylic parts) arising by the cleavage of the bond between two carbon atoms that were originally linked by a double bond, and "carboxylic-minus-methanol" ion (Dowd 2012).

Based on our preceding experience with the analysis of uncommon FAs by GC-MS, we analyzed the odd chain fatty acids. They were identified as a mixture of positional isomers of heptadecenoic acid, which was prepared by precursor directed biosynthesis using different yeast strains.

Materials and methods

Standards

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). 10-Heptadecenoic acid methyl ester was purchased from Larodan Fine Chemicals AB (Sweden).

Cultivation

The yeast precultures (*Candida* sp. DBM 2163; *Kluyveromyces polysporus* DBM 2171 (CCY 30-5-10); *Rhodotorula glutinis* CCY 20-2-20; *S. cerevisiae* DBM 2115; *Torulaspora delbrueckii* DBM 39; *Trichosporon cutaneum* CCY 30-5-10; *Yarrowia lipolytica* CCY 29-26-36) (DBM—Collection of Yeasts and Industrial Microorganisms, University of Chemistry and Technology Prague, Czech Republic; CCY—Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic) were cultured on YPD medium (20 g/L peptone, 10 g/L yeast extract, 20 g/L glucose, initial pH 6.0) in 500-ml Erlenmeyer flasks in a rotary shaker at 150 rpm at 28 °C with 200 ml YPD medium to the late exponential growth phase (26 h) and used as seed cultures to a final concentration of 0.2 A_{600}. The mineral medium composition with VFA (acetic acid 4 g/L or propionic acid 4 g/L) or glucose (20 g/L) as a sole carbon source was (in 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 (in grams per liter) $MnCl_2\ 4H_2O$ (20); $CaCl_2\ 2H_2O$ (20); $FeSO_4\ 7H_2O$ (1); $NaMoO_4\ 2H_2O$ (1). The pH was adjusted to 6.0. The yeasts were stored at 4 °C on a solid medium (5=malt extract agar)

and subcultured bi-weekly. For long-term storage stock cultures were maintained in 20 % glycerol at −60 °C.

After sterilization (121 °C for 20 min), 200 ml of mineral medium in 500-ml Erlenmeyer flasks was inoculated with 10 ml of preculture and incubated in a rotary shaker at 150 rpm and 28 °C to the stationary phase (30 h) with glucose as a carbon source or 96 h with VFA (acetic acid or propionic acid) as a sole carbon source (Rezanka et al. 2013).

Lipid extraction and isolation of total lipids

The extraction and isolation of total lipids from yeast suspension was performed according to the methodology previously described (Rezanka et al. 2013). Briefly, the yeast suspension was ground with glass beads under liquid nitrogen and the crushed yeast was extracted according to Bligh and Dyer (1959).

Analysis of fatty acid methyl esters and dimethyl disulfides

Again, FAMEs were prepared after saponification and further esterification (Rezanka et al. 2013) using BF_3/MeOH.

The FAMEs (~1 mg) were dissolved in dimethyl disulfide (0.2 mL), and a solution of iodine in diethyl ether (3 mg in 0.05 mL) was added. The mixture was stirred for 1 day, then hexane (5 mL) was added, and the mixture was washed with dilute sodium thiosulfate solution, dried over anhydrous sodium sulfate and evaporated. The products were dissolved in hexane and analyzed by GC-MS.

Gas chromatography–mass spectrometry of FAME was done on a Varian GC-MS system with the split/splitless injector (250 °C) and a SP™-2380 Capillary GC Column L×I.D. 60 m×0.25 mm, *df* 0.20 μm capillary column. Helium was used as carrier gas at 1.0 ml/min. The split/splitless injection port was maintained at 255 °C. The split ratio was 1:90, and the injection volume was 1 μL. For FAME GC-MS analysis with the SP-2380 column, the temperature program was as follows: 150 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 250 °C, which was maintained for 1 min. Higher oven temperature was used to separate the dimethyl disulfides; the starting temperature was 180 °C for 1 min, subsequently increasing at 20 °C/min to 220 °C and at 2 °C/min to 260 °C, which was maintained for 1 min. FAMEs were identified according to their mass spectra (Juzlova et al. 1996; Vancura et al. 1988; Rezanka et al. 2013) and

Table 1 Biomass, lipid, and heptadecenoic acid content in yeast strains cultivated on different substrates

Strain	Substrate	Dry mass (g/L)	Lipids in % of dry mass	lipids (g/L)	17:1 (mg/L)
Candida sp. 39	G	4.46	35.4	1.58	0.0
Candida sp. 39	A	3.15	24.2	0.76	14.5
Candida sp. 39	P	1.98	15.7	0.31	111.3
K. polysporus	G	4.51	5.1	0.23	0.2
K. polysporus	A	4.02	3.2	0.13	0.1
K. polysporus	P	3.24	2.1	0.07	22.8
R. glutinis 2185	G	3.00	33.2	1.00	0.0
R. glutinis 2185	A	2.84	19.5	0.55	1.7
R. glutinis 2185	P	1.52	14.6	0.22	41.7
S. cerevisiae 2101	G	4.16	3.8	0.16	0.2
S. cerevisiae 2101	A	1.27	2.8	0.04	0.1
S. cerevisiae 2101	P	0.31	2.4	0.01	0.4
T. delbrueckii 39	G	4.46	4.6	0.21	0.6
T. delbrueckii 39	A	3.69	3.8	0.14	0.7
T. delbrueckii 39	P	2.85	2.7	0.08	23.4
T. cutaneum	G	1.34	48.9	0.66	0.0
T. cutaneum	A	1.07	33.2	0.36	4.6
T. cutaneum	P	0.86	23.9	0.21	67.0
Y. lipolytica 2181	G	4.40	17.9	0.79	0.0
Y. lipolytica 2181	A	4.21	13.3	0.56	21.3
Y. lipolytica 2181	P	3.53	8.9	0.31	88.6

G glucose, *A* acetic acid, *P* propionic acid

Table 2 Content of individual FA in yeast strains under study in percent of total fatty acids identified by means of GC-MS of FAMEs

Strain	Source	15:0	16:0	9-16:1	17:0	8-17:1	9-17:1	10-17:1	18:0	9-18:1	11-18:1	9,12-18:2	20:0
Candida sp. 39	G	0.0±0.0[a]	16.7±0.9	8.8±0.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.3±0.2	38.0±0.8	0.6±0.2	29.7±0.9	3.9±0.2
Candida sp. 39	A	0.1±0.1	16.4±1.0	5.9±0.4	0.0±0.0	0.1±0.1	1.8±0.3	0.0±0.0	2.7±0.2	41.2±1.2	0.7±0.2	28.2±1.0	2.9±0.4
Candida sp. 39	P	1.2±0.2	7.1±0.5	1.4±0.2	0.0±0.0	24.3±0.9	0.2±0.1	11.3±0.8	2.1±0.3	26.4±0.9	0.0±0.0	23.9±0.8	2.1±0.3
K. polysporus	G	0.0±0.0	7.4±0.4	59.2±1.1	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.9±0.1	28.6±0.8	0.1±0.1	2.7±0.4	0.9±0.2
K. polysporus	A	0.1±0.1	5.2±0.4	56.6±1.2	0.2±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.8±0.3	34.3±0.9	0.4±0.2	1.4±0.3	0.9±0.3
K. polysporus	P	0.2±0.1	1.8±0.2	34.1±0.9	1.2±0.3	14.5±0.7	0.1±0.1	18.9±0.9	0.7±0.2	27.5±0.9	0.0±0.0	0.2±0.1	0.8±0.2
R. glutinis 2185	G	0.0±0.0	13.2±0.9	1.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	7.1±0.4	63.8±1.1	1.4±0.3	12.1±0.6	1.3±0.2
R. glutinis 2185	A	0.2±0.1	12.6±0.8	0.9±0.2	1.0±0.2	0.1±0.1	0.0±0.0	0.2±0.1	6.9±0.3	64.0±0.9	1.3±0.3	11.6±0.6	1.2±0.3
R. glutinis 2185	P	0.2±0.1	12.2±0.7	0.3±0.1	1.1±0.3	8.9±0.5	0.1±0.1	9.8±0.9	5.2±0.4	51.3±1.2	0.1±0.1	10.0±0.7	0.8±0.2
S. cerevisiae 2101	G	0.0±0.0	11.4±0.8	39.3±0.9	0.2±0.1	0.1±0.1	0.0±0.0	0.0±0.0	4.1±0.4	37.2±0.9	0.6±0.2	3.5±0.3	3.6±0.3
S. cerevisiae 2101	A	0.0±0.0	9.5±0.4	35.6±0.8	3.1±0.3	0.1±0.1	0.0±0.0	0.1±0.1	3.7±0.3	41.4±1.0	0.8±0.3	2.7±0.4	3.0±0.3
S. cerevisiae 2101	P	0.0±0.0	8.2±0.3	34.8±1.0	3.7±0.4	1.8±0.3	0.1±0.1	3.2±0.4	3.2±0.3	40.4±1.2	0.1±0.1	2.8±0.3	1.7±0.2
T. delbrueckii 39	G	0.0±0.0	25.0±1.1	22.6±0.8	0.7±0.2	0.2±0.1	0.0±0.0	0.1±0.1	3.6±0.2	24.0±0.7	0.4±0.2	16.8±0.9	6.6±0.4
T. delbrueckii 39	A	0.0±0.0	21.8±1.0	21.7±0.7	0.9±0.2	0.2±0.1	0.0±0.0	0.3±0.1	2.9±0.2	32.5±1.0	0.3±0.1	15.3±0.8	4.1±0.4
T. delbrueckii 39	P	0.0±0.0	14.2±0.7	18.9±0.7	2.9±0.3	14.7±0.9	0.1±0.1	15.6±0.9	3.1±0.4	14.4±0.8	0.0±0.0	14.5±0.9	1.6±0.3
T. cutaneum	G	0.0±0.0	17.0±0.8	2.6±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.8±0.3	25.9±0.9	0.2±0.1	48.8±1.0	1.7±0.2
T. cutaneum	A	0.7±0.1	16.5±0.7	2.1±0.2	0.2±0.1	0.4±0.2	0.1±0.1	0.8±0.2	2.4±0.3	29.8±0.8	0.2±0.1	45.6±1.1	1.2±0.2
T. cutaneum	P	1.2±0.2	12.7±0.5	2.1±0.3	2.1±0.2	18.2±1.3	0.2±0.1	14.2±1.0	2.1±0.3	25.0±0.8	0.0±0.0	21.2±0.7	1.0±0.2
Y. lipolytica 2181	G	0.0±0.0	13.3±0.6	8.2±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.1±0.2	47.9±1.2	0.9±0.2	24.1±0.7	4.5±0.4
Y. lipolytica 2181	A	0.7±0.1	12.4±0.5	6.9±0.4	0.6±0.2	1.1±0.2	1.0±0.2	1.7±0.2	2.5±0.3	45.5±1.1	1.0±0.2	23.2±0.5	3.4±0.3
Y. lipolytica 2181	P	0.7±0.1	9.4±0.4	3.2±0.3	1.9±0.3	13.9±0.7	0.1±0.1	14.2±1.1	1.9±0.2	31.4±0.9	0.2±0.1	21.3±0.9	1.8±0.4

[a] Arithmetic means from three analyses±standard deviations

using a mixture of chemical standards obtained from Sigma-Aldrich. All experiments concerning the analysis of FAMEs and their derivatives were carried out by electron impact MS.

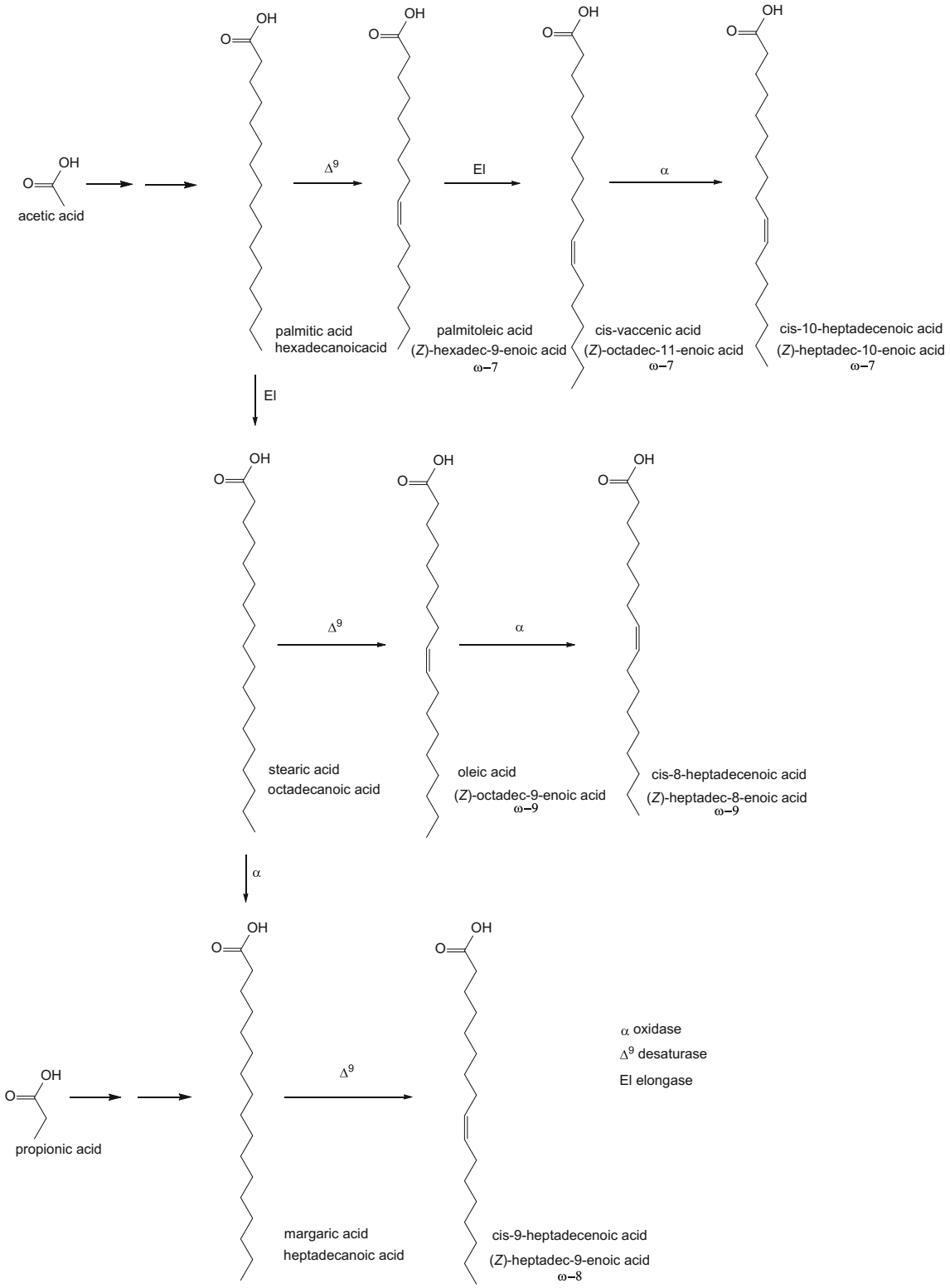

Fig. 1 Scheme of heptadecenoic acid biosynthesis

Results and discussion

Cultivation of different yeasts

We used a relatively inexpensive material as a carbon source (Lim et al. 2008), namely acetic or propionic acids (volatile fatty acids; VFA), metabolic intermediates in the fermentation step of an anaerobic digestion process that form parts of food wastes, sludge, and a variety of biodegradable organic wastes (Chang et al. 2010). Oleaginous yeasts can convert these VFAs directly into acyl-CoA, a key starter unit for the synthesis of FAs.

Our cultivations were performed in Erlenmeyer flasks, and the selection of an optimal concentration of the carbon sources, 4 g/L for both acids and 20 g/L for glucose, was based on preliminary experiments and literature data. At this concentration, both acids were utilized by the yeast. The yields of biomass, total lipids, and identified heptadecenoic fatty acid are given in Table 1. Many authors have stated that in the case of cultivation of the VFA as the sole source of C, it is required to use a lower concentration of VFA than 5 g/L. Higher concentrations inhibit growth until its complete stop (Zhao et al. 2012; Fei et al. 2011). It would seem that using fed-batch cultivation, the higher dose of VFAs can be divided into more additions, but this results in unduly prolonged cultivation times with little effect increases of the lipid content compared to batch cultures (Fontanille et al., 2012; Christophe et al., 2012). Experimental data show that very low biomass increase occurs in culture with acetic acid concentration of 5 g/L (Zhao et al. 2012; Christophe et al. 2012).

The Table 2 shows that yeasts grown on 4 g/L acetic acid or 20 g/L glucose (a cultivation considered standard) produce amounts of individual products that are in keeping with our previous results (Rezanka et al. 2013) and other literature data. Cultivation on propionic acid revealed a similar drop in the productions of biomass and total fatty acids, but a marked difference was observed in the profile of fatty acids (see below).

Studies describing cultivation of yeasts on volatile fatty acids, either as the only carbon sources or in a mixture with glucose or glycerol, are rather scant or provide equivocal data. The dry mass and the content of total lipids strongly depend on carbon source, the highest production of biomass and lipids being recorded in yeast growing on glucose as the only carbon source. Cultivation on acetic acid was found to cause a moderate decrease in both biomass and lipid content. Fontanille et al. (2012) cultivated *Y. lipolytica* in a medium with glucose or glycerol plus propionic acid; the major produced FAs were palmitic, palmitoleic, stearic, oleic, and linoleic acids accounting for about 80 % of total FAs. When acetic acid was added to glucose or glycerol as another carbon source, the content of the above acids was 90 %, and this 10 % difference may be ascribed to C17 acids, see Fig. 1 for biosynthetic pathway. This is in keeping with the findings of Zhelifonova et al. (1974) who cultivated *C. tropicalis* on lactic, butyric, and propionic acid and found, in full agreement with the fatty acid biosynthesis, that cultivation on propionic acid caused the content of C17 acids to rise from 2–4 % (determined in cultivations on lactic and butyric acid) to nearly 75 %. This included 10 % of 17:0 and 64 % of 17:1 out of total

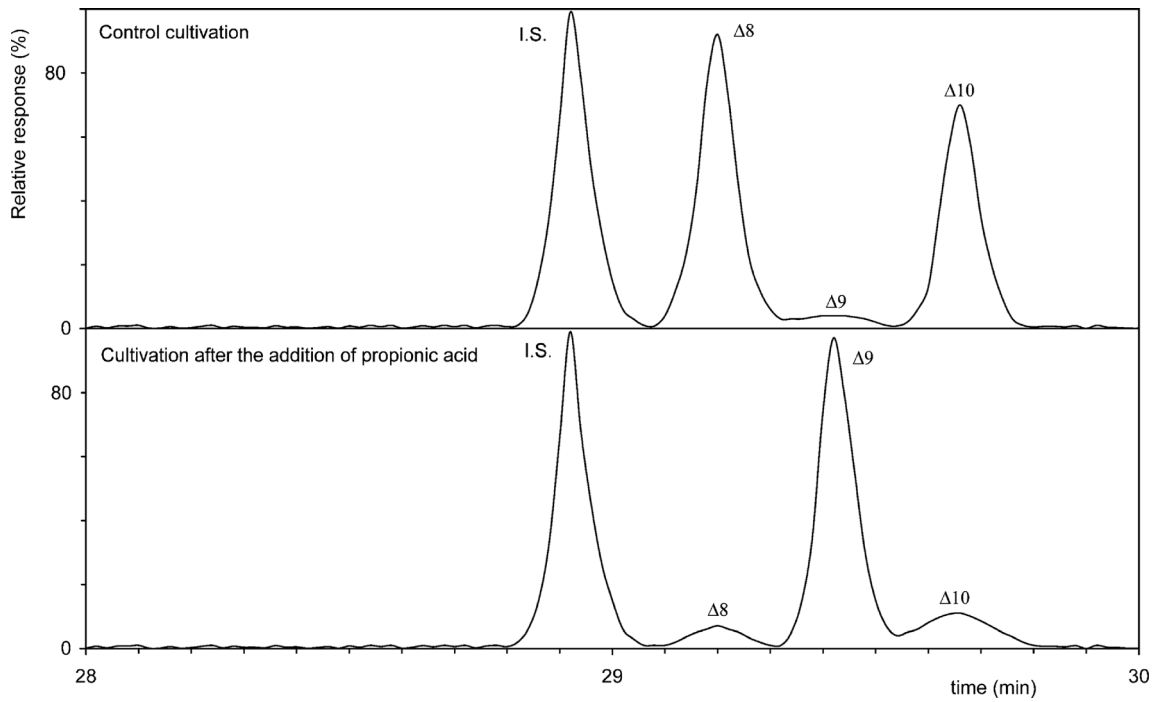

Fig. 2 Chromatogram of positional isomers of heptadecenoic acid derivatives analyzed by GC-MS (electron impact ionization; EI-MS)

FAs. We found that the production of heptadecenoic acid by *Candida* sp. cultivated on propionic acid amounted to 56 g/kg dry mass (see Table 1), an amount exceeding two times the previously determined value (24.1 g/kg dry mass) (Bauchart and Aurousseau 1980). Fei et al. (2011) found that cultivation of *C. albidus* on a VFA mixture (acetic, propionic, and butyric acid 6:1:3) resulted in a sharp drop of biomass production to less than 1/3 and a drop of lipids to 1/5 of the level seen in cultivation on glucose, but a sharp increase in the amount of linoleic acid.

Identification of odd chain fatty acids

Gas chromatography-mass spectrometry of FAMEs has shown that yeast strains cultivated on propionic acid as the only carbon source exhibit substantial changes in the spectrum of biosynthesized fatty acids. The results (see Table 2 and partially Fig. 2) show, apart from FAs commonly found in yeast after the addition of a carbon source with an even number of carbon atoms (glucose or acetic acid), such as palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2) acids, also two other peaks, that elute from the capillary column with polar stationary phase with retention times 28.9 and 29.2 minutes. In our analyses, we used CD_3-stearic acid methyl ester as internal standard, since deuterated FAMEs are known (Ecker et al. 2012) to have lower retention time than nondeuterated counterparts of the same structure. The mass spectrum of the first eluted peak (not shown) suggested the structure of heptadecanoic acid methyl ester, which was confirmed by co-chromatography with a commercially obtained standard. The mass spectra of methyl esters showed that the cluster of three peaks belonged to three positional isomers of heptadecenoic acid. Based on retention times and mass spectra, we assumed that it belongs to 8-, 9- and 10-17:1. The mass spectra exhibit an almost identical fragmentation, with molecular ion at *m/z* 282 and fragments at *m/z* 251 (M-MeO), 250 (M-MeOH), and 208 (M-74). All three peaks are present in both control cultivation and cultivation in the presence of propionic acid, albeit in totally different amounts and mutual proportions, see Fig. 2 and Table 1.

Identification of positional isomers of heptadecenoic acid involved the use of adducts with dimethyl disulfide (Christie and Han 2010), which were subsequently separated on the same column as FAMEs. The mass spectrum of the first eluting peak contained an ion at *m/z* 376 $[M]^+$ and 3 diagnostic ions, i.e. α-fragment, (α-fragment)-32, and ω-fragment at *m/z* 203, 171 and 173, respectively, see also the structure of 8-17:1 in the inset of the corresponding mass spectrum and data in Figs. 1S, 2S, and 3S (Supplements). The other peak contained major ions—fragments at *m/z* 217, 185 and 159—which point to the structure of the original FAME as 9-17:1. The third heptadecenoic acid was the positional isomer 10-17:1, as determined from the diagnostic ions at *m/z* 231, 199, and 145. This is in agreement with the order of elution of the adducts from the polar column (Dowd 2012).

Conclusions

When cultivated on propionic acid, all seven yeast species had a lower proportion of even chain FAs (16:0, 18:0, 20:0, 16:1, and 18:2) and a higher content of odd numbered FAs (15:0, 17:0, and 17:1) compared to cultivation on acetic acid or glucose. The content of oleic acid in most species was maximal in cultivations on acetic acid. Precursor-directed biosynthesis using different yeasts can thus shape the biosynthesis of fatty acids in a desirable direction. The possibilities of GC/MS analysis of derivatives of FAs in determining positional isomers of monoenoic fatty acids differing in the position of double bond are shown.

Acknowledgments The research was supported by GACR projects P503/11/0215 and P503 14-00227S, and by Institutional Research Concept RVO61388971.

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