Research Article

Polydatin and its derivatives inhibit fatty acid desaturases in microorganisms

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Antioxidant plant polyphenols resveratrol and its glycoside polydatin at 500 μg/L were found to have two distinct effects on yeasts and algae depending on the cell content of polyunsaturated fatty acids (PUFA). In yeasts that do not produce PUFA, resveratrol (0.5 mg/L) increased resistance to biosynthesized ethanol (an important property in winemaking) to 22% EtOH in *Saccharomyces cerevisiae* and 14% EtOH in *Torulaspora delbrückii*, the fatty acid content being changed only slightly, whereas in the PUFA-producing algae *Desmodesmus quadricauda* and *Euglena gracilis* both compounds act as inhibitors of Δ4 desaturases blocking the formation of both even- and odd-numbered PUFA having the first double-bond in position 4. This leads to the biotechnologically desirable accumulation of the health-beneficial polyunsaturated fatty acids (PUFAs), docosapentaenoic (DPA), and docosahexaenoic acid (DHA).

Practical applications: Employing natural stilbene compounds has far-reaching use in biotechnological applications such as the production of ethanol used for biofuels or production of wine and beer. It allows the production of beer with higher alcohol content, without resorting to the use of consumer-unacceptable genetically modified yeast. In brewing, when the yeast is often repeatedly reused to ferment fresh wort, higher resistance of yeast in the presence of these compounds can lead to an increase in the number of yeast reuses and thereby reduce economic costs of the brewery needed for preparation of inocula. Using polydatin as inhibitor of Δ4 desaturase in *Euglena* and *Desmodesmus* algae blocks the biosynthesis of both even- and odd-numbered PUFAs having a double bond in position 4. This leads to a change in the content of fatty acids and accumulation of biosynthetic precursors, e.g., 22:5n-3 acid that has more favorable pharmacological effects than docosahexaenoic acid (22:6n-3)

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1 Introduction

The number of articles on the biosynthesis of polyunsaturated fatty acids (PUFAs) is huge [1]. Biosynthesis of C18 PUFAs, that is, biosynthetic pathways based on oleic acid and proceeding over linoleic, α- or γ-linolenic to stearidonic acids are well understood [2]. Slightly less is known about the higher homologues (C20 and/or C22 PUFAs) such as arachidonic, eicosapentaenoic, and docosahexaenoic acids [3]. A totally different situation is with C16 PUFAs, for which the knowledge of their biosynthesis is very fragmentary. Freshwater algae have a similar FA composition as terrestrial plants. They differ, however, from terrestrial plants in that they contain a higher proportion of C16 PUFAs but less C18 PUFAs. Unlike marine algae, and with a few exceptions, they contain C20 and/or C22 PUFAs [4].

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Abbreviations: PUFAs, polyunsaturated fatty acids; **FAMEs,** fatty acid methyl esters

The first model organism to be tested in this respect was a photosynthetic protist *Euglena gracilis*, a commonly used and readily culturable unicellular flagellate with a highly flexible cell surface, which can grow phototrophically or heterotrophically on acetates, organic acids, alcohols, or sugars. *Euglena* species are found predominantly in freshwater habitats and under suitable conditions they can form substantial blooms. Even though *E. gracilis* is a very important research organism, it is considered to be relatively rare in nature [5].

Biosynthesis of PUFAs, especially 20:5ω-3 and 22:6ω-3, can proceed in two ways. One was described in marine bacteria such as *Shewanella, Vibrio*, or *Photobacterium* [6, 7], but also in the unicellular protist *Schizochytrium* [8]. The other, so to say classical biosynthesis, starts from linoleic acid and a series of biosynthetic steps catalyzed by elongases and desaturases take part in the biosynthesis of 22:6ω-3. These biosynthetic pathways have been described in many organisms (Fig. 1) including algae and fungi [9] but also animals such as *Caenorhabditis elegans* [10] or vertebrates including man [11], in whom however the situation is complicated by the Sprecher pathway [12], see also Fig. S1 (Supplementary Materials).

Only a few works describe the presence of C16 PUFAs in algae in higher amounts. The content of C16 PUFAs ranges up to one third of total FAs [13]. The genera *Scenedesmus* (*Desmodesmus*), *Chlamydomonas*, and *Ankistrodesmus* and other green algae also have a high content of C16 PUFAs [2]. 7,10,13-Hexadecatrienoic acid (16:3ω-3), is present in higher plants in significant amounts and is located in chloroplast glycerolipids, whereas, for example, the diatom *Phaeodactylum tricornutum* contains its isomer 6,9,12–16:3 (16:3ω-4) [14].

The 6,9,12,15-hexadecatetraenoic acid (16:4ω-1) was first identified in fish oil and phytoplankton [15], in marine and freshwater diatoms [16, 17]. Another isomer of 16:4ω-1, that is, 4,7,10,13–16:4 (16:4ω-3) is a major acid in chloroplast galactolipids of green algae *Chlamydomonas reinhardtii*, and also in related algae [13].

A significant role in explaining the biosynthesis of C18 and C20/C22 PUFAs is played by desaturase inhibitors, which are low molecular weight chemical compounds of various structures. An example is the herbicide SAN 9785, an inhibitor of chloroplastic-3-desaturation [18] used on the red alga *Porphyridium cruentum*. Another inhibitor is salicylhydroxamic acid, which was used in the same algae and which causes inhibition of Δ6 desaturation [19]. However, the most common are aromatic compounds of the type of substituted phenols, many of which come from sesame oil [20], see Fig. 2. Addition of sesame oil, which contains secondary metabolites of the type of sesamolin, sesaminol, episesamin, and especially sesamin inhibits Δ5 desaturase but not the Δ6, Δ12, or Δ9 desaturases [21].

As natural sources are not rich in 22:5ω-3 acid (DPA) due to its fast biotransformation to 22:6ω-3, blocking the biosynthesis of 22:6ω-3 is one of the possible approaches to obtaining lipids with a higher proportion of 22:5ω-3 that has been reported to have health-beneficial effects [22]. Mammalian cells, including human, metabolize 22:5ω-3 to so-called pro-resolving mediators, a specialized class of PUFA metabolites which include resolvins, protectins, and maresins. 22:5ω-3 acid inhibits platelet aggregation more efficiently than 20:5ω-3 or 22:6ω-3, stimulates endothelial cell migration much more efficiently than 20:5ω-3, and it is incorporated into human plasma and red blood cell lipids faster than 20:5ω-3 [23].

We studied the effects of another polyphenolic compound, that is, resveratrol, a known antioxidant [24, 25] on two yeast species, *Saccharomyces cerevisiae* and *Torulaspora delbrückii* as organisms that do not produce PUFA, and we found that low concentrations of resveratrol added to the yeast culture medium sharply enhance the content of ethanol during fermentation, while causing no significant changes either in qualitative or quantitative content of fatty acids. In the case of PUFA-producing algae *Desmodesmus quadricauda* and *Euglena gracilis* the situation was quite different and resveratrol and its glycoside polydatin blocked Δ4 desaturase in the biosynthesis of PUFAs, that is, FAs having the first double bond in position 4. This inhibition resulted in the accumulation of biosynthetic precursors and thereby a biotechnologically highly desirable change in the proportion of PUFAs in the algae.

2 Materials and methods

2.1 Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). 4(Z),7(Z),10

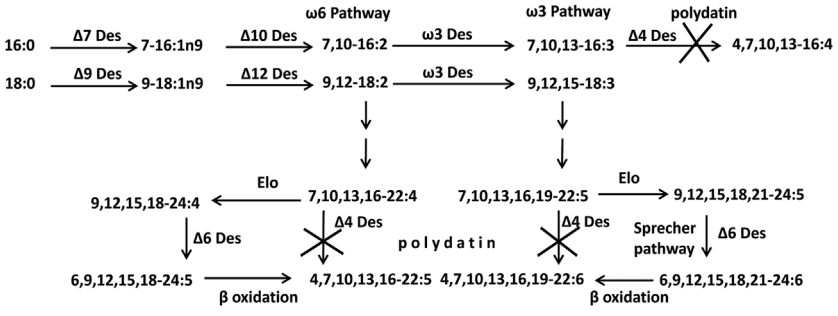

Figure 1. Scheme of PUFAs biosynthesis.

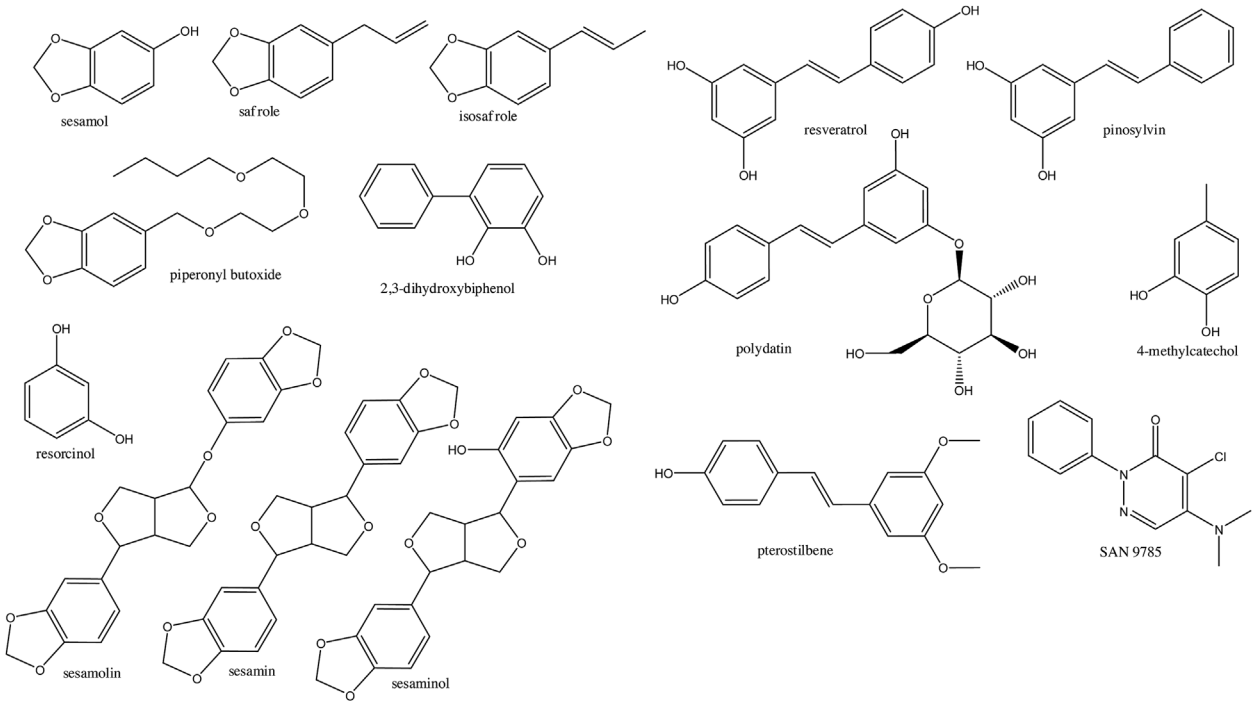

Figure 2. Structures of potential inhibitors of different fatty acid desaturases.

(Z),13(Z)-Hexadecatetraenoic acid (16:4ω-3) was purchased from Cayman Pharma, (Neratovice, Czech Republic).

2.2 Microorganisms and their cultivation conditions

2.2.1 Yeasts

Saccharomyces cerevisiae DBM 2101 and *Torulaspora delbrückii* DBM 39 were kindly provided by the Collection of Microorganisms at UCT Prague. Yeasts were pre-cultured before each experiment for 24 h (30°C, 250 mL in Erlenmeyer flasks, 100 rpm) to achieve exponential phase of growth in YEPD medium (glucose 2%, neopeptone 2%, and yeast extract 1%). The strains were stored at −70°C.

Growth characteristics were determined in the above yeast strains cultivated with different ethanol concentrations (0; 2; 4; 6; 8; 10; 12, 16, 18, 20, 22, and 24% v/v) and addition of 0.5 mg/L resveratrol and polydatin in the Bioscreen C (Labsystems, Finland) device in microtiter plates. Polydatin was dissolved in distilled water. Since the resveratrol solution was prepared by dissolving it in 40% ethanol, the volume of added ethanol was recalculated so that its desired concentration in each well was maintained. The influence of the solvent was also measured.

Cultivation temperature was 30°C, optical density was determined every 2 h. All experiments were performed five times. The cultivations were carried out for 48 h (until early stationary phase).

2.2.2 Algae

The green algae *Desmodesmus quadricauda* (Turpin) Brébisson (CCALA 453) (previously reported as *Scenedesmus quadricauda*), strain Greifswald/15, was obtained from the Culture Collection of Autotrophic Organisms, Institute of Botany (CCALA, CAS, Třeboň). The microalga was cultivated in liquid mineral medium [26], in laboratory scale tubular photobioreactors. Cultures were aerated with air containing 2% carbon dioxide v/v. Fluorescent lamps (Osram DULUX L, 55 W/840, Italy) were used as a source of the light with a surface incident irradiance of 500 μmol/m^2/s. A continuous light regime was applied. To evoke stress conditions, two suboptimal temperatures 20°C and 35°C were used for the cultivation.

Euglena gracilis Klebs, strain Pringsheim Z, was also obtained from the CCALA collection under number 349. It was cultivated in Erlenmeyer flasks in the liquid euglena medium (http://ccala.butbn.cas.cz/en/euglena-medium) at 25°C and the surface incident irradiance of 150 μmol/m^2/s 14 days. Resveratrol and/or polydatin were added in the concentration of 500 μg/L.

2.3 Lipid extraction

Lyophilized yeasts and algae were 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 three times and 50 mL of 0.1 mol/L Na_2CO_3 was finally added. The crushed cells were extracted with a chloroform-methanol mixture according to Ref. [27]. The samples were centrifuged and the lower phase was evaporated to dryness and the lipid dry weight was determined.

2.4 Analysis of fatty acid methyl esters (FAMEs) and 3-pyridylcarbinyl esters

Again, FAMEs were prepared after saponification and further esterification [28] using BF_3/MeOH. Gas chromatography-mass spectrometry of FAMEs was done on a Varian GC/MS system with the split/splitless injector (250°C) and a SLB®-IL111 capillary GC Column L × I.D. 100 m × 0.25 mm, df 0.20 μm. 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 SLB®-IL111 column, the temperature program was as follows: 150°C for 1 min, subsequently increasing at 2°C/min to 180°C and at 1°C/min to 250°C, which was maintained for 1 min. FAMEs were identified according to their mass spectra [28–30] and 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.

A solution of potassium *tert*-butoxide in tetrahydrofuran (0.5 mL, 1.0 M) was added to nicotinyl alcohol (1 mL). After mixing, the appropriate FAMEs (~0.5 mg) in dry dichloromethane (1 mL) were added, and the mixture was held at 40°C for 30 min in a closed vial. After cooling to room temperature, water, and hexane were added, and the organic phase was collected, dried over anhydrous sodium sulfate, and evaporated [31].

3-Pyridylcarbinyl ester mixture was analyzed on the instrument described above. Injection temperature (splitless injection) was 100°C, and a SLB®-IL59 Capillary GC Column L × I.D. 30 m × 0.25 mm, df 0.20 μm was used. The temperature program was as follows: 100°C for 1 min, subsequently increasing at 4°C/min to 180°C and at 2°C/min to 270°C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. All spectra were scanned within the range *m/z* 70–600.

3 Results and discussion

3.1 Cultivation of yeasts

Based on the similarity of the structure of inhibitors of Δ5, Δ6, etc. desaturases already used, see Fig. 2, that is, differently substituted polyphenols, it was assumed that polydatin and its derivatives will have similar effects as sesaminol, episesamin, and especially sesamin. We used for the cultivation two yeast species (*Saccharomyces cerevisiae* and *Torulaspora delbruckii*) that are used most commonly in winemaking. Grapes themselves contain polydatin and its related derivatives [32], and these compounds are thus a normal part of the culture medium. Cultivation of both yeasts with resveratrol and polydatin (for structural formulas see Fig. 2) revealed that the lipid content and FA composition was not significantly changed (Table 1). However, an increase in the resistance of the yeasts to higher concentrations of ethanol occurred (Figs. 3 and 4). In *S. cerevisiae*, the maximum ethanol concentration at which the cells are able to grow in the presence of the polyphenols was 24% regardless of the polyphenol added. In contrast, in the yeast *T. delbruckii*, which is commonly used for wine fermentation [33, 34], the limit growth-supporting concentration of ethanol in the presence of resveratrol and/or polydatin was a mere 14% (Figs. S1 and S2, i.e., Supplements). This finding will be further used for other biotechnological productions, particularly in winemaking. The impact of resveratrol and

Table 1. Biomass, lipid content, and FA composition of *Saccharomyces cerevisiae* cultured with inhibitors (mean ± S.D. from three measurements).

Cultivation	Dry weight (mg/100 mL)	Lipids (mg/100 mL)	% lipids in dry weight	14:0	16:0	16:1ω-7	18:0	18:1ω-9	18:1ω-7	20:0
Control	196	9.6	4.9	2.5 ± 0.2	19.2 ± 0.7	40.8 ± 1.1	5.1 ± 0.4	28.7 ± 1.2	0.5 ± 0.1	3.2 ± 0.3
Resveratrol 500 μg/L	172	9.0	5.2	2.9 ± 0.3	17.7 ± 0.5	37.6 ± 1.0	5.8 ± 0.5	31.7 ± 1.0	0.6 ± 0.1	3.7 ± 0.2
Resveratrol 5000 μg/L	168	7.1	3.9	2.7 ± 0.2	18.0 ± 0.8	39.1 ± 0.9	5.8 ± 0.6	29.7 ± 0.9	0.7 ± 0.2	4.0 ± 0.4
Polydatin 500 μg/L	178	8.3	4.7	2.6 ± 0.4	17.3 ± 0.6	38.0 ± 1.4	5.1 ± 0.5	32.7 ± 1.3	0.1 ± 0.1	4.2 ± 0.2
Pinosylvin 500 μg/L	179	9.3	5.2	2.3 ± 0.3	26.7 ± 0.9	36.5 ± 0.8	4.3 ± 0.4	25.9 ± 1.2	0.4 ± 0.2	3.9 ± 0.3
Pterostilbene 500 μg/L	174	9.5	5.5	2.8 ± 0.2	21.2 ± 0.8	38.4 ± 1.5	5.0 ± 0.7	28.4 ± 1.4	0.4 ± 0.1	3.8 ± 0.3

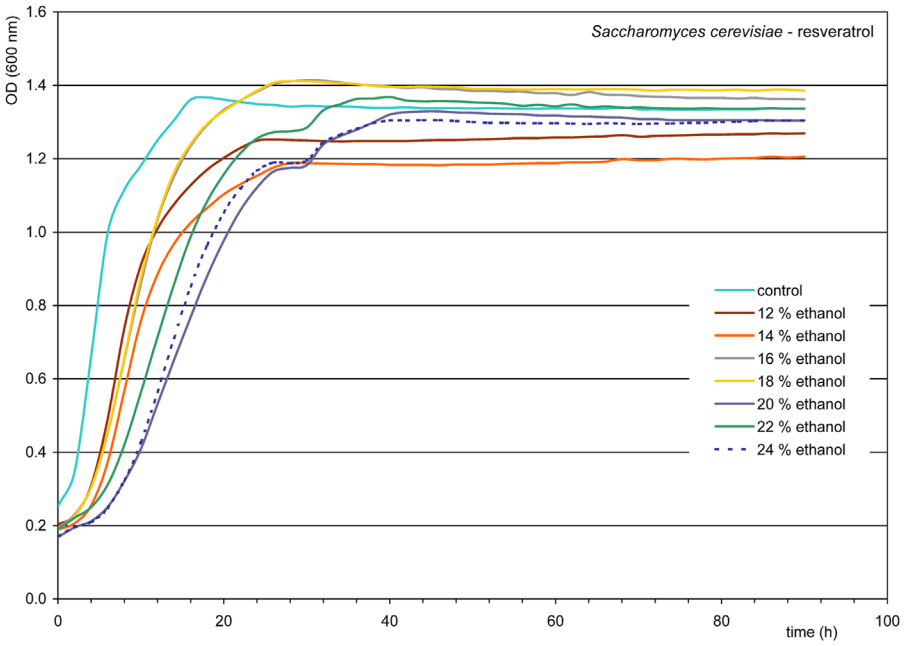

Figure 3. Growth curves of yeast *Saccharomyces cerevisiae* with different concentrations of polydatin.

polydatin on both studied yeasts is quite logical because neither of them produces PUFAs, as neither was found to produce appropriate desaturases necessary for the production of PUFAs. This of course holds for wild yeast, not genetically modified yeast. The above results extend the findings on the complicated mechanisms of ethanol tolerance of cells. These include transcription factor, whose production is stimulated by ethanol activated signaling pathways, nucleotide sequences, and stress response elements to which the transcription factors are bound [35, 36]. Other parts of cell response include antioxidant mechanisms by which cells respond to increased amounts of reactive oxygen species [37], stabilization of proteins [38] and adjustment of the composition of the cytoplasmic membrane [39].

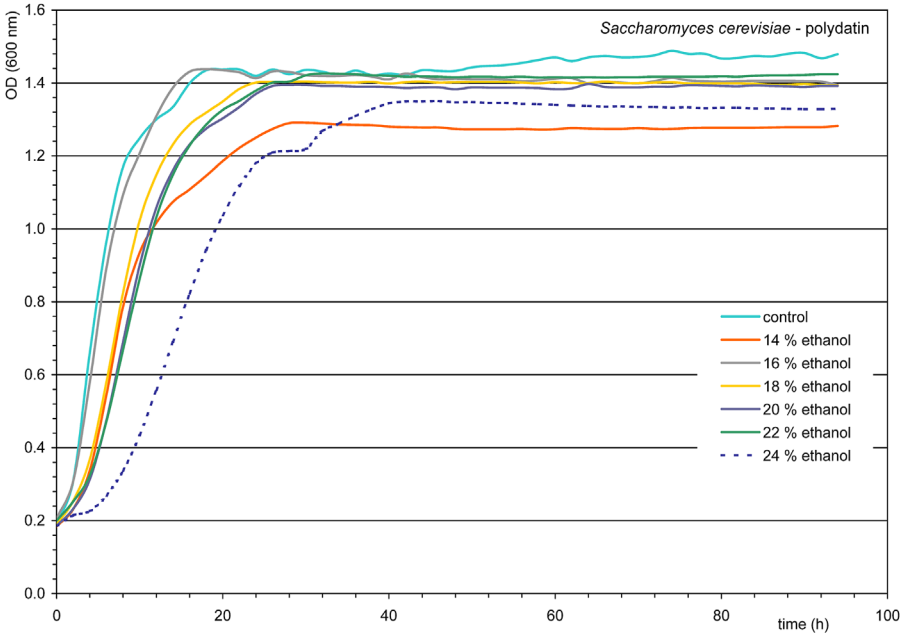

Figure 4. Growth curves of yeast *Saccharomyces cerevisiae* with different concentrations of resveratrol.

3.2 Cultivation of algae

3.2.1 Determination of structures of PUFAs

Briefly, PUFAs were tentatively identified based on GC/MS spectra of methyl esters separated on a polar capillary column. Identification of positions of double bonds was performed on the basis of the fragmentation described above; the ion at 150 Da abundant in the mass spectrum is characteristic for ω-6 PUFAs whereas the ion at 108 Da for ω-3 PUFAs [40]. Full identification was performed again by GC/MS with the aid of 3-pyridylcarbinol esters which always contain molecular ions with odd values. Typical in mass spectra are also ions at 92, 108, 151 (the McLafferty ion), and 164 Da. The molecular ion may lose a methyl group (M-15 ion) and the spectrum also displays a series of ions with interval decrements of 14 Da reaching up to the first double bond from the terminal methyl of the molecule. The 14 Da interval is then replaced by a 26 Da or 40 Da "gap" interval. An example is the identification of two positional isomers of 3-pyridylcarbinol docosapentaenoic esters; all spectra are illustrated in Figs. S3 and S4.

3-Pyridylcarbinol ester of 22:5ω-3 acid (Fig. S4) showed a gap of 26 Da between *m*/*z* 392.3 and 364.2, which locates the double bond in position 19 while the gap between *m*/*z* 352.2 and 326.2 locates double bond in position 16. It is however easier to spot gaps of 40 Da for the double bond and an associated methylene group, that is gaps from *m*/*z* 206. 1–246.1 to 286.2–326.2 to 366.2. The spectra of our compound (pyridylcarbinol ester of 22:5ω-3) and the same substance shown on the website [41] are very similar. This clearly implies the structure of 22:5ω-3.

An analogous approach was adopted with the positional isomer, that is, 22:5ω-6 (Fig. S3). The mass spectra show differences between the two pyridylcarbinol esters, that is, different values of the 26 Da and/or 40 Da gaps. The gap of 26 Da between *m*/*z* 350.2 and 324.2 locates the double bond in position 16 while the gap between *m*/*z* 310.2 and 284.2 locates a double bond in position 13, etc. More diagnostic were the 40 Da gaps which showed the following values: 164.1–204.1 to 244.1–284.2 to 324.2–364.2 Da. Based on all measured values—retention time, the presence of ion at 150 Da in methyl ester and mass spectrum of 3-pyridylcarbinol ester—this compound was identified as 22:5ω-6 acid. These facts clearly show that these compounds are positional isomers of C22 PUFAs, as also indicated by the agreement of the above mass spectra with the spectra published on web pages [40]. Other acids were identified in a similar way as methyl esters and also 3-pyridylcarbinol esters.

All FAs had a *Z* (formerly *cis*) geometric configuration of all double bonds since the IR spectrum was not found to show absorption of the C-H bond in the range of 960–980 cm^{-1} or more precisely at 967 cm^{-1}, which belongs to the *E* (formerly *trans*) double bond(s) [42].

3.2.2 Biosynthesis in *Euglena gracilis*

As shown above, we assumed that polydatin and its derivatives, like other compounds (see Fig. 2), inhibit desaturases of FAs and thus also the biosynthesis of PUFAs.

For several reasons, *E. gracilis* was chosen as a suitable model for the study of the biosynthesis of PUFAs and of selected inhibitors based on the structure of polydatin and related compounds (Fig. 2). The content of 22:6ω-3 in *Euglena* amounts to units of per cent [43, 44]. In the cases when the content of this acid was found to be higher, such as in *Thraustochytrium*, *Schizochytrium*, or the dinoflagellate *Crypthecodinium,* it was not clear if the 22:6ω-3 acid derives from the biosynthesis by polyketide synthase or whether it results from a very efficient mutual exchange of intermediates by PUFAs desaturases and elongases such as those found in *Chlorococcum* or *Isochrysis* [13].

One of the fundamental works that have been published in this area describes Δ4 desaturases from *E. gracilis* and *Osteococcus lucimarinus* which were cloned to *S. cerevisiae* and surprising substrate specificity was found [45]. Three PUFAs (16:3ω-3, 22:4ω-6, and 22:5ω-3) were added to the culture medium of the cloned yeast. The desaturases from both algae have approximately the same enzymatic activity in desaturation of C22 PUFAs. However, desaturase from *O. lucimarinus* was practically ineffective when it was transforming 16:3ω-3 to 16:4ω-3 [45]. This is another reason why the basic experiments were performed only with the protist *Euglena* and not with green algae of the genus *Desmodesmus*. Furthermore, the Δ4 desaturase of *E. gracilis* was cloned into *S. cerevisiae* [46] and its sequence is thus known.

Δ4 desaturases were also identified in other algae—for example, in *Isochrysis galbani* and *Pavlova lutheri*, from which the corresponding genes were cloned to *S. cerevisiae*. Upon adding exogenous 22:4ω-6 or 22:5ω-3, the yeast produced 22:5ω-6 and 22:6ω-3 PUFAs, respectively [47, 48].

In addition, the number of FAs identified in *E. gracilis* including odd-numbered FAs is in excess of 50 (Table 2), which points to the diversity of biosynthetic pathways. Based on these findings and on published data [46] we conducted the cultivation of *E. gracilis*.

E. gracilis was cultivated with two concentrations of resveratrol and its glycoside polydatin. Our results show that the activity of Δ4 desaturase is negligibly affected by different concentrations of the agents and also by whether the agent is either the aglycone resveratrol or its glycoside polydatin. The inhibition by 500 μg/L resveratrol is given in Table 2 and Fig. 5. The figure clearly shows that the addition of the inhibitor caused a drop in the biosynthesis of all PUFAs having a double bond in position Δ4. Identification of both starting PUFAs (7,10,13,16–22:4, i.e., 22:4ω-6, and 7,10,13,16,19–22:5, i.e., 22:5ω-3) and the products of biosynthesis, that is, 4,7,10,13,16–22:5 and 22:5ω-6 and 4,7,10,13,16,19–22:6, that is, 22:6ω-3 acids was also

Table 2. FA composition of *Euglena gracilis* cultured with resveratrol and polydatin (concentration 500 μg/L) (mean ± S.D. from three measurements).

FA		Control	Resveratrol	Polydatin
12:0		0.49 ± 0.15	0.46 ± 0.09	0.45 ± 0.14
13:0		0.30 ± 0.11	0.28 ± 0.06	0.28 ± 0.11
14:0		11.63 ± 0.89	10.91 ± 1.58	10.69 ± 1.64
5–14:1		0.93 ± 0.09	0.87 ± 0.21	0.85 ± 0.23
n15:0		0.47 ± 0.08	0.44 ± 0.12	0.43 ± 0.14
7–14:1		0.23 ± 0.05	0.22 ± 0.09	0.21 ± 0.09
9–14:1		0.12 ± 0.04	0.11 ± 0.05	0.11 ± 0.05
16:0		8.14 ± 1.24	7.64 ± 1.32	7.48 ± 1.11
7–15:1		0.81 ± 0.09	0.76 ± 0.17	0.74 ± 0.17
9–15:1		0.35 ± 0.07	0.33 ± 0.15	0.32 ± 0.14
16:1[a]		0.18 ± 0.06	0.17 ± 0.08	0.17 ± 0.08
17:0		1.16 ± 0.22	1.09 ± 0.22	1.07 ± 0.24
7–16:1		1.14 ± 0.19	1.07 ± 0.28	1.05 ± 0.25
9-16:1		1.23 ± 0.22	1.15 ± 0.24	1.13 ± 0.22
4,7–15:2	ω-8	0.29 ± 0.07	0.27 ± 0.09	0.27 ± 0.13
18:0		2.28 ± 0.34	2.14 ± 0.34	2.09 ± 0.42
7,10–15:2	ω-5	0.58 ± 0.11	0.53 ± 0.14	0.53 ± 0.13
9–17:1		0.58 ± 0.24	0.55 ± 0.18	0.53 ± 0.15
7,10–16:2	ω-6	3.49 ± 0.54	3.28 ± 0.75	3.21 ± 0.36
15:3[a]		0.05 ± 0.03	0.42 ± 0.13	0.52 ± 0.17
9–18:1		2.33 ± 0.89	2.19 ± 0.42	2.14 ± 0.42
11–18:1		0.23 ± 0.08	0.22 ± 0.08	0.21 ± 0.09
7,10–17:2	ω-7	0.71 ± 0.12	0.66 ± 0.13	0.64 ± 0.16
4,7,10,13–15:4	ω-2	0.69 ± 0.13	0.11 ± 0.05	0.08 ± 0.05
9,12–17:2	ω-5	0.23 ± 0.07	0.22 ± 0.07	0.21 ± 0.08
7,10,13–16:3	ω-3	3.49 ± 0.76	7.64 ± 0.86	7.48 ± 0.97
11–19:1		0.23 ± 0.12	0.22 ± 0.13	0.21 ± 0.07
4,7,10,13–16:4	ω-3	8.14 ± 1.47	3.28 ± 0.57	3.21 ± 0.35
9,12–18:2	ω-6	4.30 ± 0.64	4.04 ± 0.64	3.95 ± 0.46
9,12,15–17:3	ω-2	1.75 ± 0.24	1.64 ± 0.24	1.61 ± 0.27
9,12–19:2	ω-7	0.12 ± 0.04	0.11 ± 0.04	0.11 ± 0.06
11,14–19:2	ω-5	0.23 ± 0.06	0.22 ± 0.09	0.21 ± 0.10
9,12,15–18:3	ω-3	9.89 ± 0.99	9.28 ± 1.42	9.09 ± 1.34
17:4[b]		0.12 ± 0.04	0.11 ± 0.05	0.11 ± 0.06
11,14–20:2	ω-6	2.21 ± 0.53	2.07 ± 0.36	2.03 ± 0.41
19:3[b]		0.05 ± 0.03	0.05 ± 0.03	0.05 ± 0.03
18:4[b]		0.12 ± 0.07	0.11 ± 0.06	0.11 ± 0.05
5,8,11,14–19:4	ω-5	0.70 ± 0.24	0.66 ± 0.15	0.64 ± 0.22
8,11,14–20:3	ω-6	1.28 ± 0.35	1.20 ± 0.24	1.18 ± 0.37
11,14,17–20:3	ω-3	2.09 ± 0.28	1.96 ± 0.32	1.92 ± 0.21
5,8,11,14–20:4	ω-6	6.98 ± 0.72	6.55 ± 0.53	6.41 ± 0.65
8,11,14,17–20:4	ω-3	2.33 ± 0.36	2.19 ± 0.41	2.14 ± 0.28
5,8,11,14,17–20:5	ω-3	4.65 ± 0.48	4.36 ± 0.32	4.27 ± 0.61
7,10,13,16–21:4	ω-5	0.35 ± 0.13	0.33 ± 0.10	0.32 ± 0.14
7,10,13,16–22:4	ω-6	1.37 ± 0.17	4.96 ± 0.66	6.21 ± 0.39
4,7,10,13,16–22:5	ω-3	4.65 ± 0.56	3.83 ± 0.41	3.67 ± 0.41
7,10,13,16,19–22:5	ω-3	2.98 ± 0.47	8.00 ± 0.89	8.69 ± 0.59
4,7,10,13,16,19–22:6	ω-3	3.33 ± 0.35	1.10 ± 0.36	0.97 ± 0.21

[a]Position of the double bonds has been identified only in culture with inhibitors.
[b]Position of the double bonds was not detected.

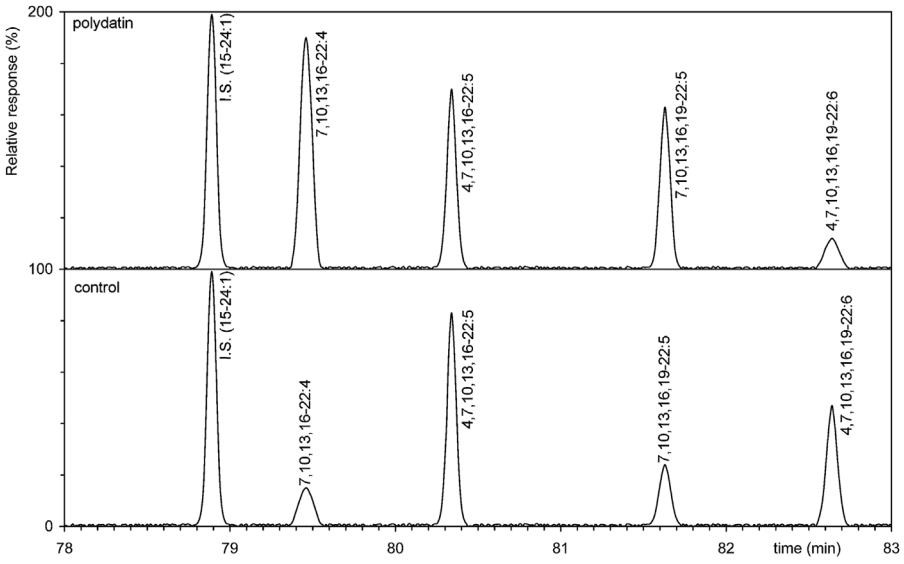

Figure 5. GC/MS (total ion current) of C22 PUFAs from control cultivation and cultivation with polydatin (*Euglena gracilis*).

confirmed by GC/MS analysis of 3-pyridylcarbinol derivatives.

Another finding pointed to the inhibition of the biosynthesis of both types of fatty acids, that is, both ω-6, and ω-3 PUFAs (i.e., 4,7,10,13,16–22:5 and 4,7,10,13,16,19–22:6). This clearly demonstrates that Δ4 desaturase acts from the terminal carboxyl. This fact is logical in view of the distance of the Δ4 double bond from the carboxyl, which is substantially smaller than that from the other end of the molecule, that is, the methyl group. We also found that the effect of inhibitors on the biosynthesis and thus also the blocking of Δ4 desaturase exhibits a broad substrate specificity independently of whether the inhibitor is the glycoside or the aglycone. The extents of inhibition were mutually comparable (Table 2). This broad specificity has of course affected the blocking of less common (minor) PUFAs such as odd-numbered fatty acids. Although their amount produced by *E. gracilis* is an order of magnitude lower than the amount of even-numbered PUFAs, we succeeded in showing that the biosynthesis of the minor odd-numbered

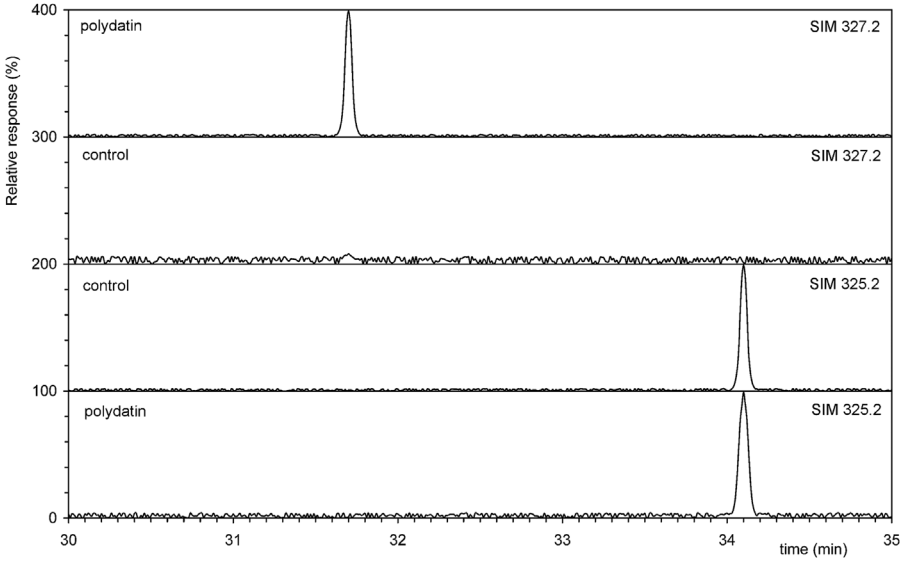

Figure 6. GC/MS (selected-ion monitoring at 325.2 and 327.2 Da) of C15 PUFAs from control cultivation and cultivation with polydatin (*Euglena gracilis*).

Table 3. Biomass, lipid content, and FA composition of *Desmodesmus quadricauda* cultured with inhibitors (both at 500 μg/L) at two temperatures (mean ± S.D. from three measurements).

Cultivation	Dry weight (mg/100 mL)	Lipids (mg/100 mL)	% lipids in dry weight	16:0	16:1ω-7	16:3ω-3	16:4ω-3	18:1ω-9	18:2ω-6	18:3ω-3	Minority FA[a]
Control 20°C	112	17	15.2	25.3 ± 1.4	0.0 ± 0.0	4.6 ± 0.4	17.3 ± 1.2	8.2 ± 1.2	2.7 ± 0.3	41.3 ± 2.4	0.6 ± 0.2
Polydatin 20°C	105	19	18.1	23.1 ± 0.9	0.8 ± 0.2	16.5 ± 0.8	4.6 ± 0.7	9.4 ± 0.8	2.7 ± 0.2	42.6 ± 2.8	0.3 ± 0.1
Resveratrol 20°C	107	20	18.7	28.5 ± 1.2	0.9 ± 0.3	19.0 ± 0.4	5.0 ± 0.5	16.7 ± 1.3	3.3 ± 0.4	26.0 ± 1.6	0.6 ± 0.2
Control 35°C	127	31	20.5	36.6 ± 1.6	3.0 ± 0.5	5.8 ± 0.5	8.3 ± 0.9	24.7 ± 1.5	4.7 ± 0.5	16.3 ± 1.2	0.6 ± 0.3
Polydatin 35°C	121	28	19.8	33.6 ± 1.4	1.3 ± 0.2	11.3 ± 0.9	6.2 ± 0.6	24.9 ± 1.6	7.6 ± 0.6	13.9 ± 1.4	1.2 ± 0.4
Resveratrol 35°C	124	24	20.2	32.5 ± 1.1	1.1 ± 0.3	11.3 ± 1.0	3.0 ± 0.4	30.8 ± 1.1	6.6 ± 0.5	13.8 ± 0.9	0.9 ± 0.3

[a]Minority FA, that is, i16:0, 3(*E*)-16:1, 7–16:1, 18:0

PUFA 4,7,10,13–15:4 in *E. gracilis* was inhibited, see Fig. 6. Consistent with published data [44], in control cultivation the biosynthetic precursor was found in a trace concentration, the corresponding 7,10,13–15:3 acid being completely identified after adding the inhibitor. The position of double bonds was again determined by GC/MS of 3-pyridylcarbinol esters (Figs. S5 and S6).

3.2.3 Biosynthesis in *Desmodesmus quadricauda*

The second model organism was the green unicellular alga *Desmosdesmus quadricauda* (Chlorophyta, Chlorococcales), earlier known as *Scenedesmus quadricauda*. The genus *Desmodesmus* was separated from *Scenedesmus* [49] and then *D. quadricauda* from closely related *Desmodesmus communis* [50]. This planktonic fresh water algae forms coenobia typically consisting of four or eight cells, is easy to grow in cultures and is therefore used for physiological, cell cycle, and toxicity studies.

The algae *Chlamydomonas reinhardtii*, the entire genome of which is known [51], was found to lack polyketide synthase. Since *D. quadricauda* belongs to the same class as C. *reinhardtii*, we can assume Δ4 desaturase would be present in this species, as it produces C16 PUFAs as well (Figs. S7 and S8). This assumption has proved to be correct (see below).

Meyer et al. [46] asserted that one of the possible substrates may be a C16 PUFA, that is, 7,10,13–16:3 acid. Apart from other FAs, this acid is produced by *D. quadricauda* [13, 52]. Table 3 shows the composition of FAs in both control cultivation and after the addition of the inhibitor. Two C16 PUFAs, 7,10,13–16:3 and its biosynthetic product—4,7,10,13–16:4 were identified by GC/MS as 3-pyridylcarbinol esters. Mass spectra were identified as in C22 PUFAs and are illustrated in Fig. S9 and S10. Table 3 again clearly shows that addition of both inhibitors, that is, resveratrol and polydatin, caused a sharp drop in the biosynthesis and hence the production of 4,7,10,13–16:4.

4 Conclusions

The use of inhibitors in yeast cultivations (see section 3.1.) is highly useful and has far-reaching biotechnological applications such as the production of ethanol used for biofuels or production of wine and beer. It makes it possible to produce beers with higher alcohol content without resorting to the consumer-inacceptable use of genetically modified yeast.

When using polydatin and resveratrol in algae, that is, in the species *Euglena* and *Desmodesmus* that we examined, it was found that the use of inhibitors of Δ4 desaturases blocks the formation of both the even-numbered (4,7,10,13–16:4, 4,7,10,13,16–22:5, and 4,7,10,13,16,19–22:6) and odd-numbered (4,7,10,13–15:4) PUFA having the first double-bond in position 4, which leads to accumulation of the biosynthetic precursors—7,10,13–16:4, 7,10,13,16–22:5, and 7,10,13,16,19–22:6, or 7,10,13–15:4. The above modifications of biosynthetic pathways can thus be used to alter the spectrum of produced fatty acids by means of purely natural substances, which resveratrol and polydatin undoubtedly are.

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