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Review

Characterization of membrane-bound fatty acid desaturases

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Abstract. Membrane-bound desaturases play key role in metabolism of polyunsaturated fatty acids. Characterization of these enzymes and their genes is the first step in basic understanding of their proper functioning in living cells as well as in tailor-made preparation of highly-specific and highly-productive strains of microorganisms interesting for applied biotechnology. It is also the crucial step in creation of transgenic agricultural crops with enhanced content of individual polyunsaturated fatty acids. Properties and applications of identified membrane-bound desaturases genes and enzymes are discussed in this review.

Key words: Desaturase — Polyunsaturated fatty acid

Abreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DGLA, dihommo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; FAS, fatty acids synthase; GLA, γ-linolenic acid; His, histidine; LA, linoleic acid; PKS, polyketide synthase system; PUFAs, polyunsaturated fatty acids; SDA, stearidonic acid.

Introduction

Polyunsaturated fatty acids (PUFAs) play important roles as structural components of membrane phospholipids and precursors of eicosanoids of signalling molecules, including prostaglandins, thromboxanes and leukotriens (Spector 1999; Jump 2002). They are important in both medical and pharmaceutical fields, being involved in the human inflammatory response, reproductive functions, immune response, blood pressure regulation, cholesterol metabolism, and infant retinal and brain development (Horrobin 1992; Benatti et al. 2004).

Edible oils for human consumption are predominantly comprised of six common fatty acids: palmitic, palmitoleic, stearic, oleic, linoleic (LA) and α-linolenic acid (ALA) (Qiu and Meesapyodsuk 2009). Human body is able to convert fatty acid precursors from these substrates to both PUFA ω-6 and ω-3 classes such as γ-linolenic acid (GLA), dihommo-γ-linolenic acid (DGLA), arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, the affectivities of these conversions are very poor especially for ω-3 PUFAs. Certain fish oils can be rich source of PUFAs although processed marine oils are generally undesirable as food ingredients because of the associated objectionable flavours and contaminants that are difficult and cost- prohibitive to remove (Damude and Kinney 2007). PUFAs are currently produced from fish or cultivated microorganisms, but their high production cost and diminishing feedstock limit their supply and usage (Chen et al. 2006). To obtain a more suitable source of large-scale production of PUFAs, the study of PUFA's biosynthetic pathway and its subsequent genetic manipulation is challenging alternative.

Therefore, the main purposes of desaturases studies can be summarized as follows:

- preparation of highly specific oleaginous microorganisms that are suitable for fermentation production
- obtaining special plants with desired composition of PUFA.

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Biosynthesis of PUFA

Biosynthesis of PUFA is associated with both membrane-bound desaturase and elongation enzymes. Figure 1 represents the aerobic biosynthetic pathway of PUFAs. The first double bond is introduced into Δ9-position of saturated fatty acid, thus palmitoleic (C 16:1, n-7) and oleic (C 18:1, n-9) are the most common monoens in organisms. Oleic acid is then, in general, transformed by Δ12-desaturase to yield linoleic acid (LA; C 18:2, n-6), which may be further converted by Δ15 (ω-3)-desaturase to α-linolenic acid (ALA; C 18:3, n-3). Thus, these fatty acids are the basic precursors of n-6 and n-3 fatty acid cascades. Next step is desaturation of these fatty acids by Δ6-desaturase. LA in n-6 pathway is converted to γ-linolenic acid (GLA, C 18:3, n-6) and ALA in n-3 pathway is converted into stearidonic acid (SDA; C 18:4, n-3). GLA and SDA are subsequently elongated in C20 fatty acid. GLA is elongated into dihommo-γ-linolenic acid (DGLA, C 20:3, n-6) and SDA into eicosatetraenoic acid (ETA, C 20:4, n-3). There occurs another desaturation step using Δ5-desaturase. In n-6 arachidonic acid (ARA, C 20:4, n-6) is synthesized and in n-3 pathway EPA (C 20:5, n-3) is synthesized. These two fatty acids go under elongation step to create C22 fatty acids with corresponding number of double bonds. Last step is catalyzed by Δ4-desaturase and docosapentaenoic acid (DPA; C 22:5, n-6) and docosahexaenoic acid (DHA, C 22:6, n-3) are produced. Each fatty acid desaturase introduces a double bond at the specific position of acyl chain (Los and Murata 1998). The Δ9-desaturase is the only universally spread desaturase being present in all living beings groups. The remaining desaturases are missing in some of the evolutionary lineages (Alonso 2003). These enzymes are spread into various types of organisms and only the certain groups of them contain each of them. The Δ12- and Δ15-desaturase occurs only in marine bacteria and algae, cyanobacteria, zygomycetes fungi and higher plants. This distribution made LA and ALA 'essential fatty acids' for animal and Δ9-desaturase essential for life (Alonso et al. 2003). The Δ6- and Δ5-desaturases are present only in marine bacteria and algae, zygomycetes fungi and animals. The Δ4-desaturase was identified only in marine

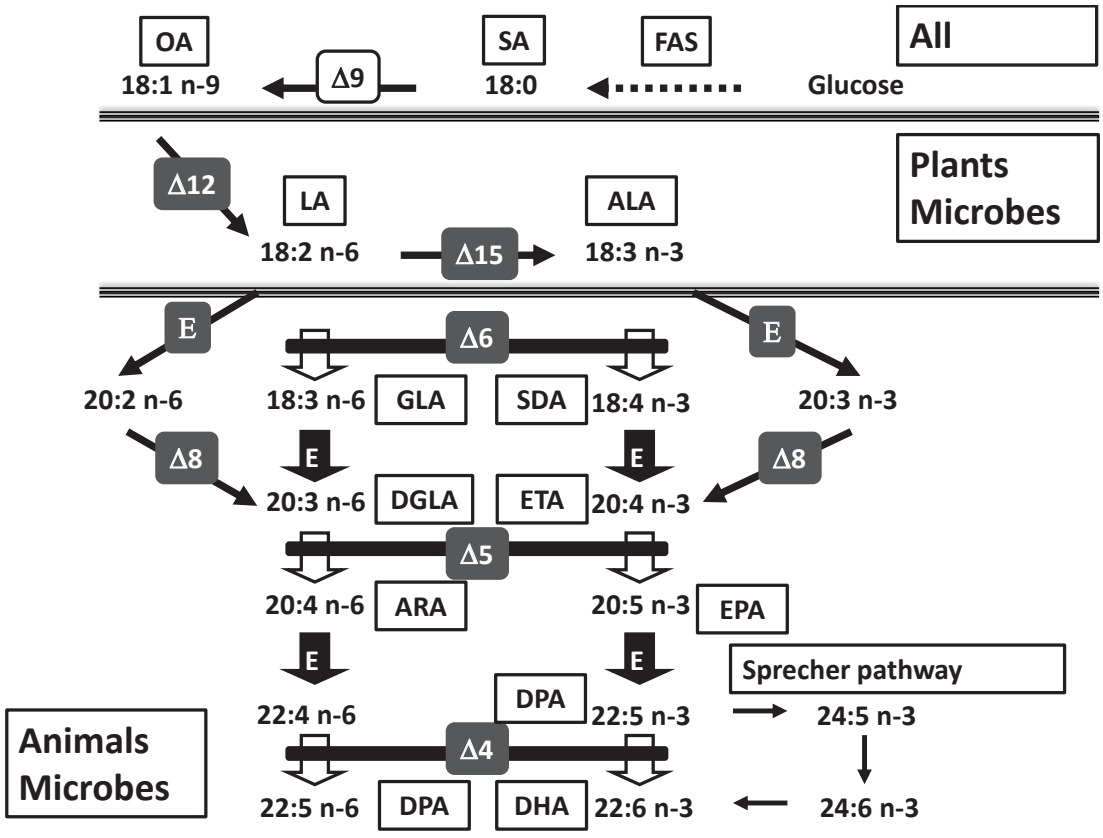

Figure 1. Aerobic biosynthetic pathway of PUFAs. FAS, fatty acid synthase system; SA, stearic acid; OA, oleic acid; LA, linoleic acid; ALA, alpha linolenic acid; GLA, gamma linolenic acid; SDA, stearidonic acid; DGLA, dihommo-gamma linolenic acid; ETA, eicosatetraenoic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid (n-6 & n-3); DHA, docosahexaenoic acid; E, elongase; ΔX, desaturation enzyme (the number express the carbon position in acyl chain where desaturation occurs). For more detailed description of pathway, see the text.

Table 1. Comparison of certain type of fatty acid desaturases

Desaturase	Cytochrome b_5 domain	1st His-box	2nd His-box	3rd His-box
Δ4	N-terminal	HXXXH	HXXXHH	QXXHH
Δ5	N-terminal	HXXXH	HXX(X)HH	QXXHH
Δ6	N-terminal	HXXXH	HXXHH	QXXHH
Δ9	C-terminal	HXXXXH	HXXHH	HXXHH
Δ12	does not contains	HXXXH	HXXHH	HXXHH

protists, algae and thraustochytrids such as *Thraustochytrium sp.* (Qiu et al. 2001), *Thalassiosira* (Tonon et al. 2005). Mammals use for last step "Sprecher pathway" instead of direct desaturation of C 22:5, n-3 to DHA. Sprecher pathway has three distinct step – first is elongation to 24:5, n-3 followed by desaturation with Δ6-desaturase and then one β-oxidation step to DHA. In the late 90's the alternative Δ8-pathway has been discover in some protists and algae species e.g. *Euglena* (Wallis and Browse 1999) or *Isochrysis* (Qi et al. 2002). This pathway involved the Δ9-elongation step of LA or ALA as first one followed by Δ8-desaturation to yield DGLA (in n-6 cascade) or ETA (in n-3 cascade).

PUFA are synthesised through both anaerobic and aerobic pathway. The desaturation system of the aerobic pathway is composed of three proteins: NAD(P)H-cytochrome b_5 reductase, cytochrome b_5 and the terminal cyanide-sensitive desaturase (Figure 2) (Certik and Shimizu 1999). The exception could be found in organisms from prokaryota group since they contain ferredoxin instead of cytochrome b_5. The desaturation takes place in the endoplasmatic reticulum where fatty acid bound to phospholipids (especially phosphatidylcholine) are desaturated rather than thiol CoA ester (Pereira et al. 2003). Membrane-bound desaturases introduce double bond into fatty acids that are either esterified as acyl CoA or bound to the glycerol moiety of glycerolipids (Los and Murata 1998).

Anaerobic biosynthetic pathway of PUFA is carried out by specialized polyketide synthase (PKS) sometimes called FAS II system (fatty acid synthase II). FAS II is a complex of four discrete enzymes each with activities equivalent to FAS I system that is common in every cell. FAS I start with condensation of acetyl-CoA with malonyl-ACP by the beta-ketoacyl-ACP synthase (KS). This is followed by the reduction of the beta-ketoester by a NADPH-dependent beta-ketoacyl-ACP reductase, the removal of water by the beta-hydroxyacyl-ACP dehydrase to produce trans-2 enoyl-ACP and another reduction by the enoyl-ACP reductase to form a saturated acyl-ACP that in turn can initiate another cycle of condensation with malonyl-ACP, reductions and dehydration. The process continues up to the synthesis of C 16-18 saturated acyl-ACPs. The shunt is produced by the action of a specific b-hydroxyacyl-ACP dehydrase that, in addition, has a trans-2-cis-3 isomerase activity on trans-2-decenoyl-ACP that results in formation of cis-3-decenoyl-ACP. Repetion of these steps results in synthesis of PUFA such as DHA. This pathway can be found in marine bacteria such as *Shewanella* or *Photobacterium*, and thraustochytrids such as *Szichochytrium* (Uttaro 2006). This strain is also an example of organisms that has possibility to synthesise PUFAs by anaerobic together with aerobic pathways.

Fatty acid desaturases

Membrane-bound desaturase have three conserved separated histidine (His) boxes and four transmembrane domains. His residues are assumed to be ligands for iron ions that are essential for catalytic place of desaturase. From topological point of view, each desaturase crosses the membrane four times in two hydrophobic domains and all three His boxes are on membrane of endoplasmatic reticulum towards cytosol (Stuckey et al. 1990; Shanklin and Cahoon 1998). This corresponds to suppose that His

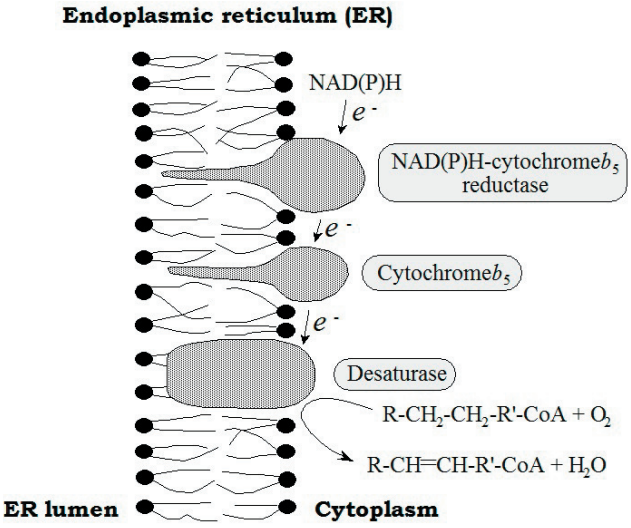

Figure 2. Desaturation system of aerobic biosynthesis of PUFAs (according to Certik and Shimizu 1999).

boxes are the catalytic places for desaturation. Sperling et al. (2003) published review that identifies two groups of desaturases that differ in number of amino acids between 1st and 3rd His box. Experimental data obtain for acyl-lipid desaturase from *Bacillus subtilis* shown that this desaturase contains another hydrophobic domain (Diaz et al. 2002). On the other hand, data concerning topology of mouse stearoyl-coA desaturase shows that this enzyme contains only two hydrophobic regions (Man et al. 2006). Author suggested that the topology of each desaturase is dependent on form of the substrate - if the fatty acid is bound in phospholipids or if it is soluble as fatty acyl-CoA.

The cytochrome b_5 domain is an essential part of functional desaturase protein. The sequence that represents this domain is HPGG. It serves as a donor of electrons for introduction the double bond in to the fatty acid chain. Any disruption of this domain results in inactivation of desaturase (Sayanova et al. 1997). Depends on certain type of desaturase, it appears on either N-terminal or C-terminal end of desaturase. The reason of deletion of this domain in Δ12-desaturase is still unknown.

The His boxes are specific for each type of desaturase and their usual motifs are: $H(X)_{3\text{-}4}H$, $H(X)_{2\text{-}3}HH$ and $H/Q(X)_2HH$ (Table 1). The 3rd His box of Δ4-, Δ5- and Δ6-desaturase rather contains glutamine then His. The 1st His box of Δ9-desaturase contains four amino acid residues between histidines rather than two. The 2nd His box of Δ4-desaturase contains three amino acids residues between histidines even the other proteins contains two amino acids. The 2nd His box of Δ5-desaturase contains typically two amino acid residues between His residues, but in some organisms is possible to found three amino acids residues between His residues.

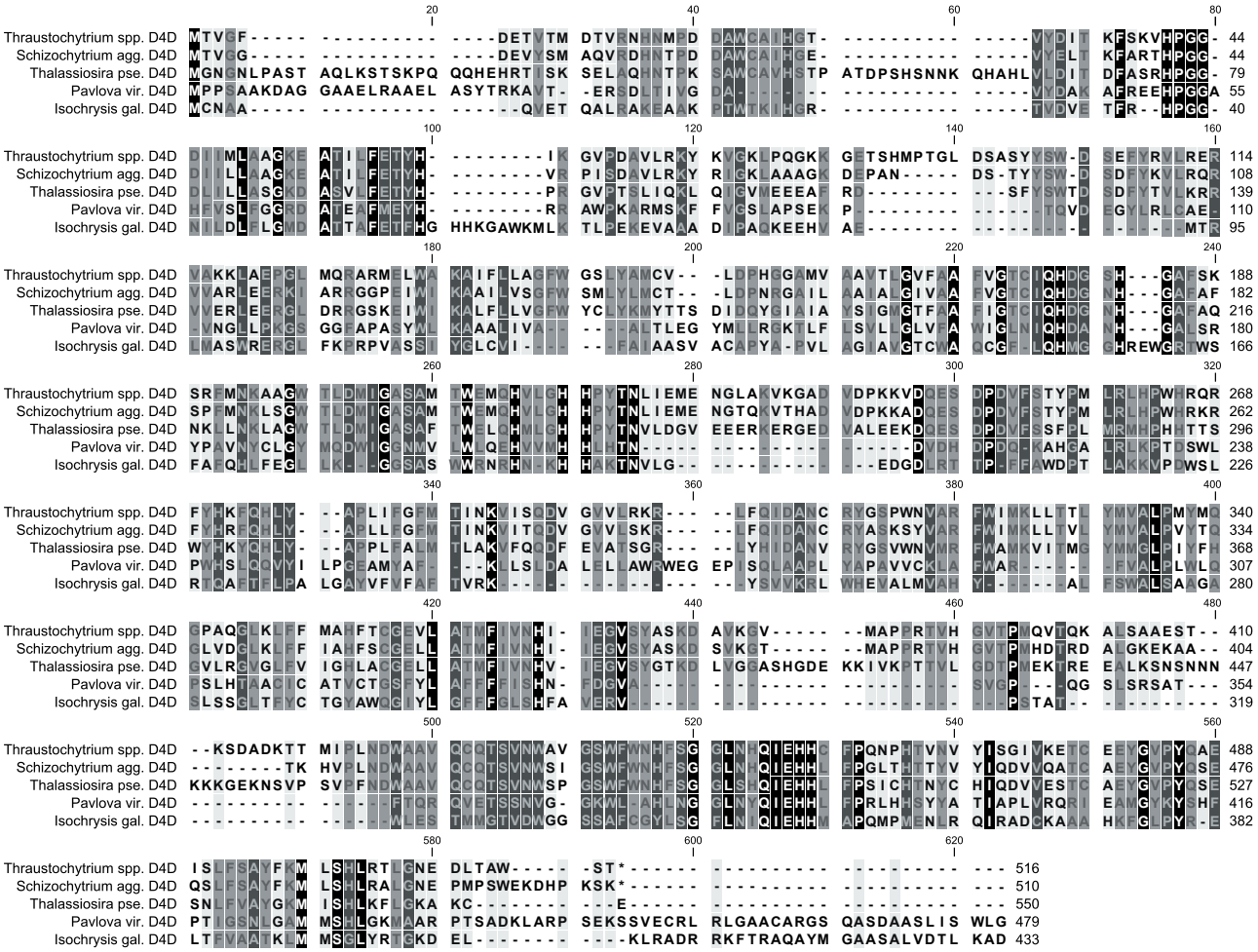

Figure 3. Alignment of amino acid sequences of Δ4-desaturase proteins (D4D) from following sources: *Thraustochytrium spp., Schizochytrium aggregatum, Thalassiosira pseudonana, Pavlova viridis* and *Isochrysis galbana*. Cytochrome b_5 domain and His box are framed, degree of sequences conservation rises from light grey up to black (black, total conservation; dark grey, high conservation; middle grey, partial conservation; light grey, low conservation). For more details see Table 1.

These changes suggest that 'front-end' desaturases has one common ancestor and through the evolution divide to three independent enzymes.

Δ4-desaturase

Δ4-desaturase is enzyme that is responsible for biosynthesis of docosapentaenoic acid (DPA C 22:5, ω-6) and DHA (C 22:6, ω-3). It was isolated from *Thraustochytrium* sp. (Qui et al. 2001; Zank et al. 2005), *Schizochytrium aggregatum* (Kinney et al. 2004), *Isochrysis galbana* (Pereira et al. 2004), *Thalassiosira pseudonana* (Tonon et al. 2005), *Pavlova viridis* (Xu et al. 2011). It has typical sign for 'front-end' desaturase – Q in 3rd His box instead of H, cytochrome b_5 domain on N-terminal, but in 2nd His box contains one more amino acid residue (Fig. 3).

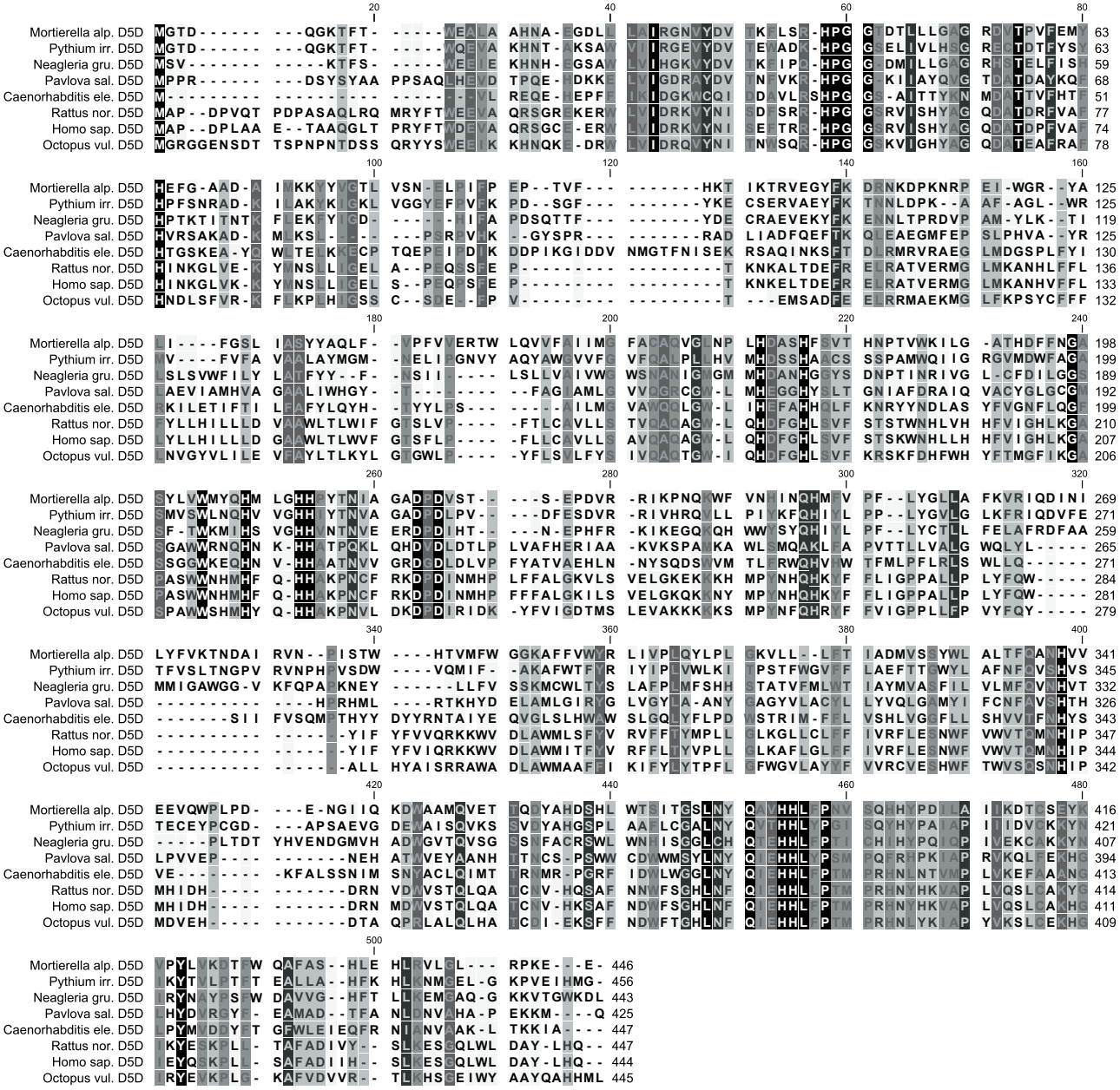

Figure 4. Alignment of amino acid sequences of Δ5-desaturase proteins (D5D) from *Mortierella alpina, Pythium irregulare, Naegleria gruberi, Pavlova salina, Caenorhabditis elegans, Rattus norvegicus, Homo sapiens, Octopus vulgaris*. Cytochrome b_5 domain and His box are framed, degree of sequences conservation rises from light grey up to black (black, total conservation; dark grey, high conservation; middle grey, partial conservation; light grey, low conservation). For more details see Table 1.

Δ5-desaturase

Δ5-desaturase catalyzes the conversion of DGLA (C 20:3, ω-6) to ARA (C 20:4, ω-3) and the biosynthesis of EPA (C 20:5, ω-3). The genes for Δ5-desaturase have been isolated from many organisms e.g. *Caenorhabditis elegans* (Michaelson et al. 1998), various strain of *Mortierella alpina* (Tavares et al. 2011), *Rattus norvegicus* (Zolfaghari et al. 2001), *Pavlova saliva* (Zhou et al. 2007), *Naegleria gruberi* (Fritz-Laylin et al. 2010), *Pythium irregulare* (Hong et al. 2002a), *Homo sapiens* (Cho et al. 1999a), *Octopus vulgaris* (Monroig et al. 2011) etc. Figure 4 shows the alignment of these sequences. This type of desaturase contains cytochrome b_5 domain located on N-terminal (typical motif HPGG). It contains three His box with

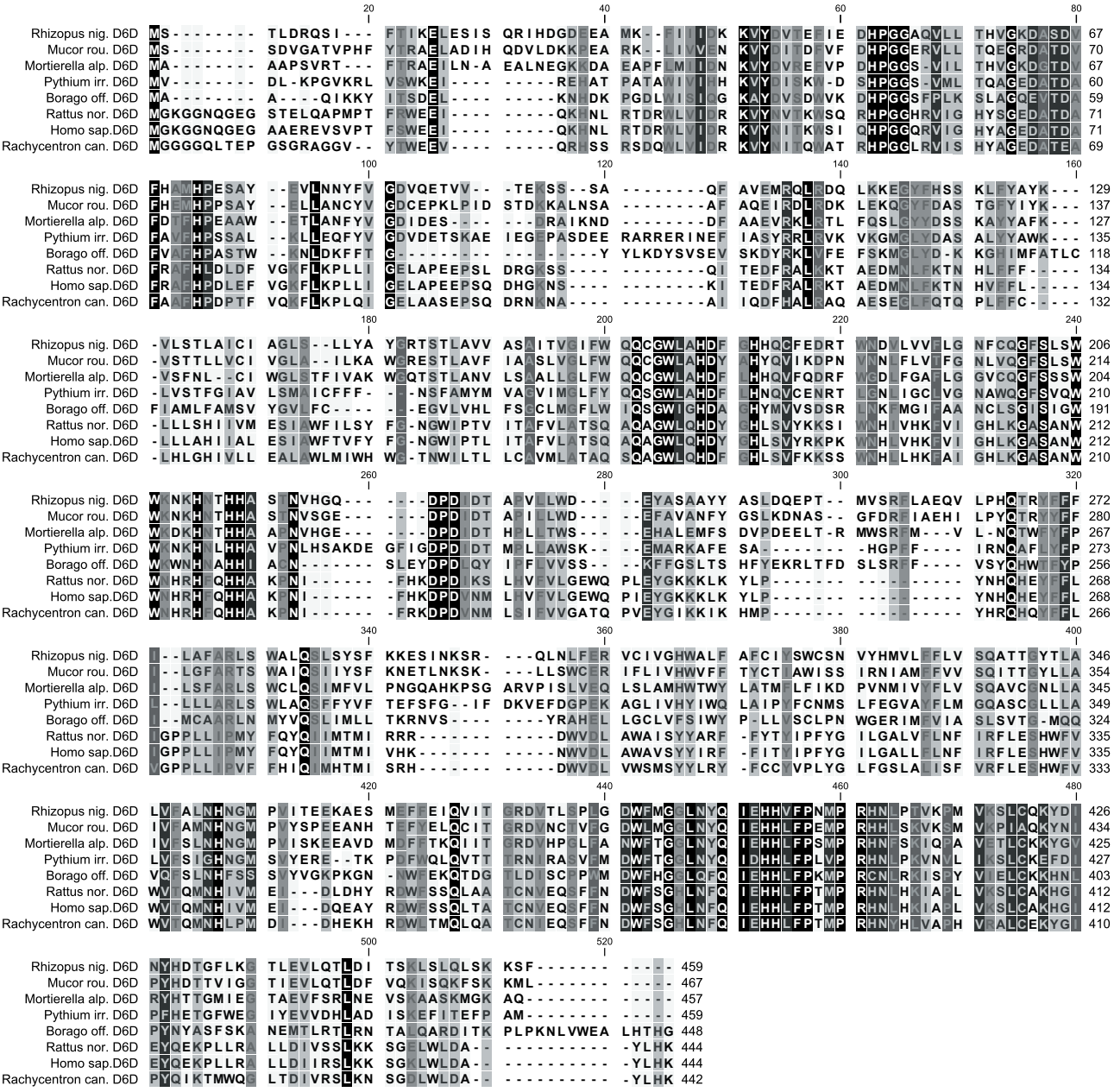

Figure 5. Alignment of amino acid sequences of Δ6-desaturase proteins (D6D) from *Rhizopus nigricans, Mucor rouxii, Mortierella alpina, Pythium irregulare, Borago officinalis, Rattus norvegicus, Homo sapiens, Rachycentron canadum*. Cytochrome b_5 domain and His box are framed, degree of sequences conservation rises from light grey up to black (black, total conservation; dark grey, high conservation; middle grey, partial conservation; light grey, low conservation). For more details see Table 1.

typical patterns. The alignment pointed out that genes from multicellular organisms (*Rattus, Homo, and Octopus*) contain the short sequence right after the start codon. It suggests that these are the signal sequences that direct the protein to the specific cell organ. Expression of Δ5-desaturase gene from *M. alpina* in transgenic canola led to production unusual fatty acid such as taxoleic (C 18:2- 5, 9) and pinoleic acid (C 18:3- 5, 9, 12) that are products of conversion of oleic and linoleic acids with Δ5-desaturase (Knutzon et al. 1998).

Δ6-desaturase

Δ6-desaturase catalyzes the conversion of LA to GLA (C 18:3, ω-6) and conversion of ALA (C 18:3, ω-3) to

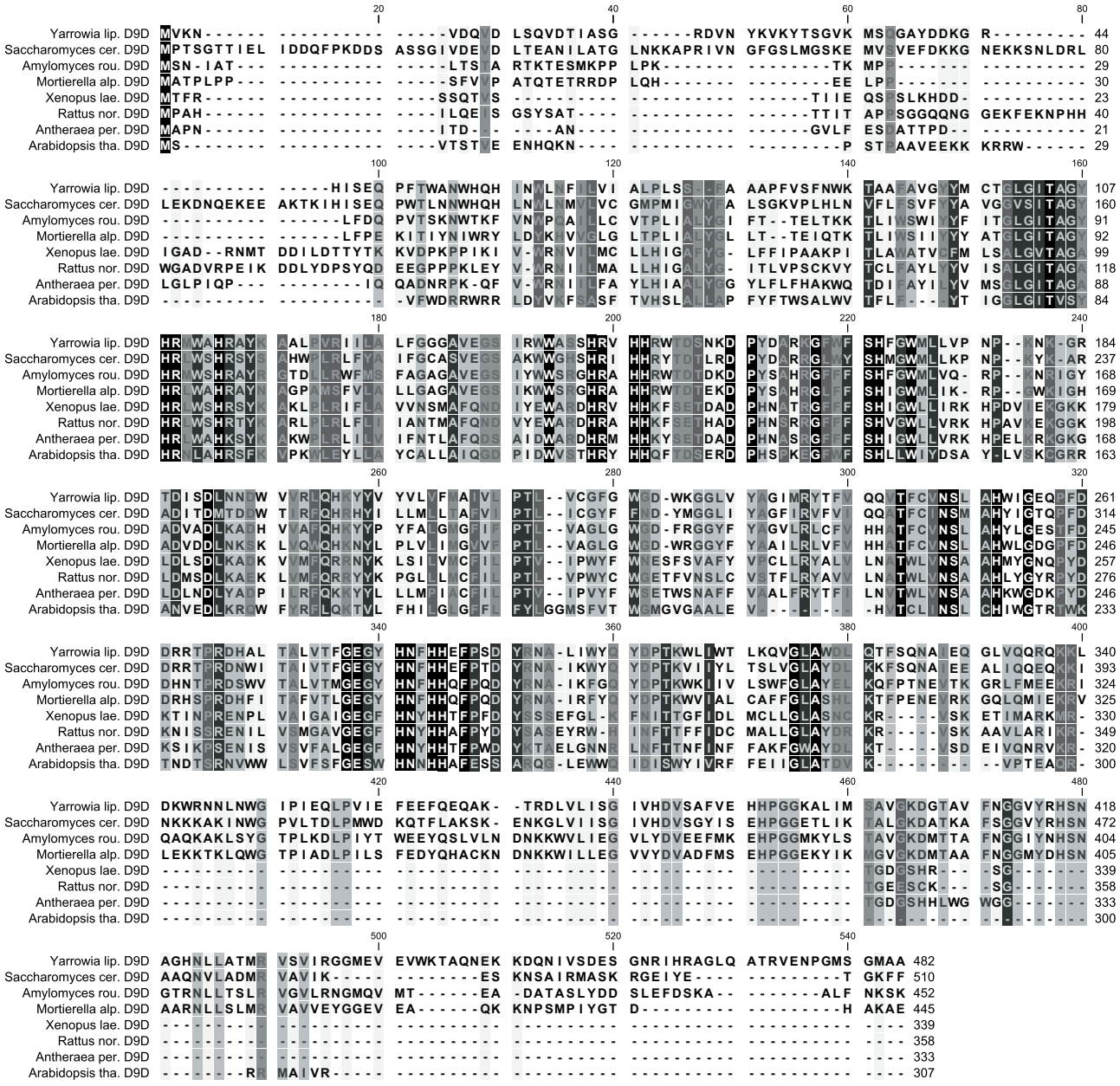

Figure 6. Alignment of amino acid sequences of Δ9-desaturase proteins (D9D) from *Yarrowia lipolytica, Saccharomyces cerevisiae, Amylomyces rouxii, Mortierella alpina, Xenopus laevis, Rattus norvegicus, Antheraea pernyi, Arabidopsis thaliana*. Cytochrome b_5 domain and His box are framed, degree of sequences conservation rises from light grey up to black (black, total conservation; dark grey, high conservation; middle grey, partial conservation; light grey, low conservation). For more details see Table 1.

stearidonic acid (SDA, C 18:4, ω-3). Many genes coding this enzymes have been isolated and characterized e.g. *Pythium irregulare* (Hong et al. 2002b), *Rhizopus nigricans* (Lu et al. 2009), *Rachycentron canadum* (Zheng et al. 2009), *Mucor rouxii* (Laoteng et al. 2005b), *Borago officinalis* (Sayanova et al. 1997), *Mortierella alpina* (Sakuradani et al. 1999a), *Rattus norvegicus* (Aki et. al 1999), *Homo sapiens* (Cho et al. 1999b) etc. The partial sequences form *Thaminidium elegans* and various *Mucor* strains have been characterized in our laboratory (GenBank accession No. HM856138, HQ437169, HQ437170, HQ437171, HQ437172). The alignment of these sequences is presented on Figure 5. Each desaturase has the cytochrome b_5 domain present on N-terminal and three His boxes with common pattern – Q instead of H in 3rd His box, two amino acid residues between His residues in 2nd His box. The change of Q to H in 3rd His box leads to complete loss of activity of protein (Sperling et al. 2003). Expression of Δ6-desaturase gene from *Cunnighamela echinulata* in yeast strain *Pichia pastoris* led to production of γ-linolenic acid with yield 3% of total lipids and octadecadienoic acid (C 18:2, ω-9) in trace concentration. However, the most controversal changes were observed in other fatty acid that occurs naturally in this yeast strain. The content of heptadecenoic acid (C 17:1) increased 30 times, and the yield of α-linolenic acid (C 18:3, ω-3) was enhanced from 3 to 15% of total lipids. On the other hand, the striking decrease was observed in linoleic acid where its content dropped from 16 to 0.1% of total lipids (Xia et al. 2009). The expression of the very same gene isolated from *Thamnidium elegans* in the same host led only to production of γ-linolenic acid and there were no significant changes in content of other fatty acids (Wang et al. 2007). Expression of Δ6-desaturase gene from *Mucor rouxii* in the yeast *Saccharomyces cerevisiae* caused the desaturation of all available fatty acids without regard to chain length. This desaturase shows no substrate specificity (Na-Ranong et al. 2005). Zhou et al. (2006) carried out the heterelogic expression of Δ6-desaturase gene from plant *Echium plantagineum* to tobacco and *Arabidopsis* that currently synthesize linoleic and α-linolenic acids. It is noteworthy that while the gene expression in *Arabidopsis* led to desaturation of both fatty acids, transformed tobacco preferentially transformed linoleic acid to GLA. These results indicate that the Δ6-desaturase specificity varies and depends not only on the origin of the gene, but also on the host. The further studies on substrate specificity of Δ6-desaturase may bring more light to the genetically-mediated production of desired PUFAs.

Δ9-desaturase

Δ9 is the only desaturase that is spread through all type of organisms. In the nature it widely catalyses the conversion of stearic acid (C 18:0) to oleic acid (C 18:1, ω-9) as well as palmitic acid (C 16:0) to palmitoleic acid (C 16:1, n-7). The enzyme was probably the most important for maintenance of proper membrane fluidity of living organismsm during the evolution. Wongwathanarat (1999) reported that the yeast Δ9-desaturase activity, compared with other fatty acid desaturases, was mostly involved to changing the membranes fluidity during temperature shift. Hsieh and Kou (2005) support this hypothesis with experiment with two fishes *Ctenopharyngodon* idella and *Chanos* chanos. Plenty of genes coding this enzyme have been isolated and characterized in other various organisms e.g. *Mortierella alpina* (Sakuradani et al. 1999b), *Amylomyces rouxii* (Laoteng et al. 2005a) *Saccharomyces cerevisiae* (Stuckey et al. 1990), *Yarrowia lipolytica* (Xue et al. 2009), *Arabidopsis thaliana* (Fukuchi-Mizutani et al. 1998), *Rattus norvegicus* (Baba et al. 1994), *Xenopus laevis* (Klein et al. 2002), *Antheraea pernyi* (Wang et al. 2010) etc. Figure 6 represents the alignment of these sequences where the cytochrome b_5 domain is located at C-terminal end of protein. Genes from multicelluar organisms – arabidopsis, rat, oak and frog do not contain this domain. The reason of the cytochrome b_5 domain deletion from Δ9-desaturases is still unknown, but in a fact it was found only in the higher eukaryotes, whew diffusible cytochrome b_5 of the endoplasmic reticulum serves as electron donor in these cases (Uttaro 2006). Figure 7 also shows that the pattern of the His boxes corresponds to typical motif of Δ9-desaturase as follows: 1st His box contains four amino acid residues between His residues and 3rd His box contains H instead of Q. Expression of the Δ9-desaturase commonly yielded to higher amounts oleic acid. Nevertheless, Kajiwara et al. (2000) interestingly reported that overexpression of Δ9-desaturase protein in *Saccharomyces cerevisiae* increased the ethanol fermentation. Also, heterological expression of Δ9-desaturase in the tomato enhanced its resistance against powdery mildew (Wang et al. 1998).

Δ12-desaturase

Δ12-desaturase is enzyme that is responsible for conversion of oleic acid to linoleic acid. This enzyme is not presented in human or any animals and that makes linoleic acid essential for human health. It has been isolated from various type of organisms such as *Mortierella alpina* (Sakuradani et al. 1999c), *Amylomyces rouxii* (GenBank No. AF533361), *Aspergillus flavus* (GenBank No. AY280867), *Chlorella vulgaris* (Suga et al. 2002), *Borago officinalis* (GenBank No. AF074324), *Helianthus annus* (Martinez-Rivas et al. 2001), *Caenorhabditis elegans* (Peyou-Ndi et al. 2000), *Trypanosoma brucei* (Petrini et al. 2004) etc. The motif of each His boxes does not display any exception from the typical pattern (Fig. 7). However, as it was mentioned above, the Δ12-desaturase does not contain the cytochrome b_5 domain. The reason for deletion of the cytochrome b_5 domain has not

been explained yet. Since the Δ12-desaturase is enzyme that needs to accept the electrons for proper function, microsomal cytochrome b_5 might be involved to this process.

Peyou-Ndi et al. (2000) heterologically expressed Δ12-desaturase gene isolated from *Caenorhabditis elegans* into *Saccharomyces cereviase*. The transformed yeasts increased (by 40%) conversion rate of palmitoleic and oleic acids to their consequent analogues with another double bond in cis-12-position. It should be noted that this transformed strain showed higher resistance to ethanol and oxidative stress.

Phylogenetic tree of fatty acid desaturases

The phylogenetic tree of chosen fatty acids desaturases (Fig. 8) indicate following interesting fact:

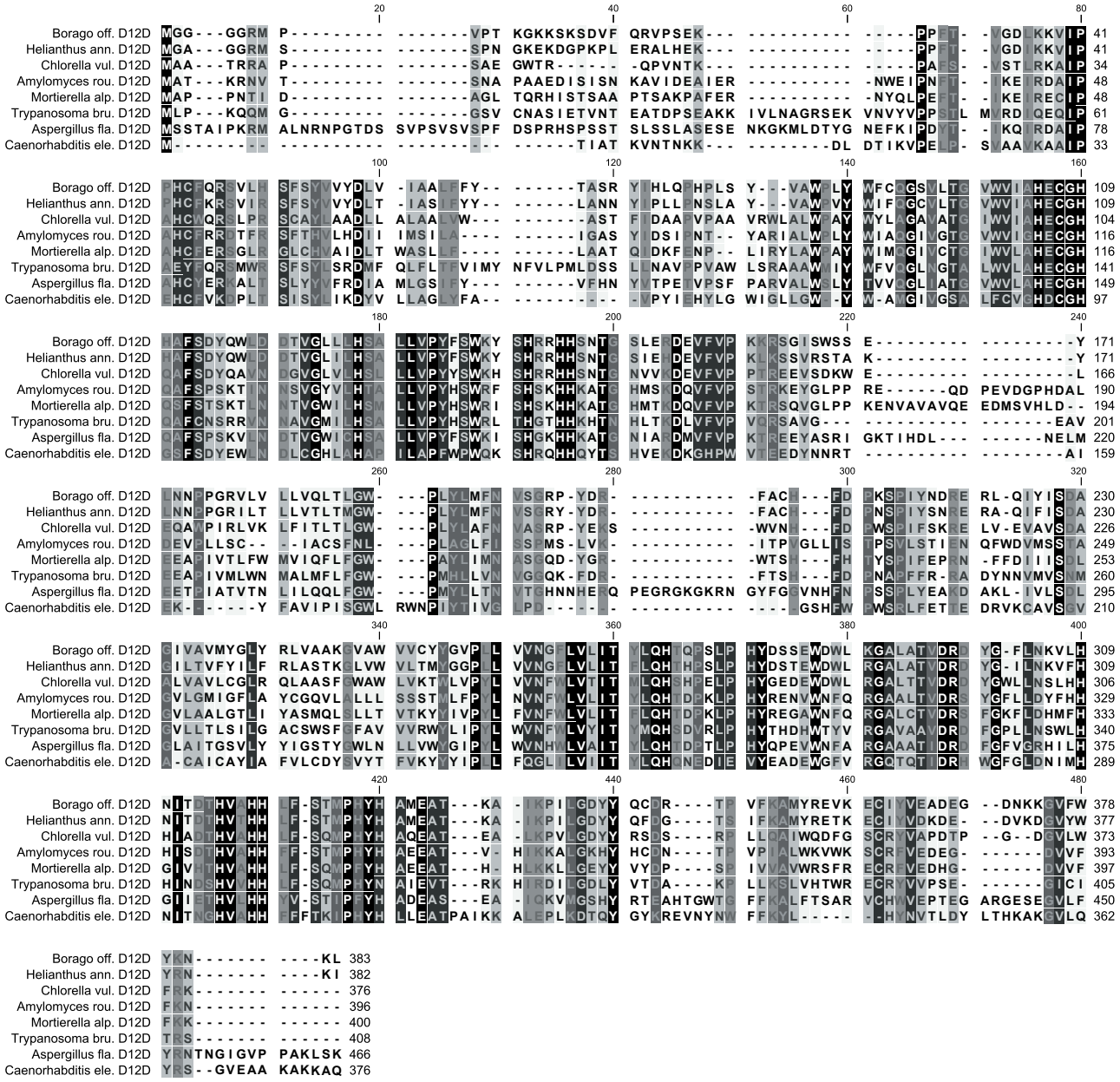

Figure 7. Alignment of amino acid sequences of Δ12-desaturase proteins (D12D) from *Borago officinalis, Helianthus annuus, Chlorella vulgaris, Amylomyces rouxii, Mortierella alpina, Trypanosoma brucei, Aspergillus flavus, Caenorhabditis elegans.* His box are framed, degree of sequences conservation raises from light grey up to black (black, total conservation; dark grey, high conservation; middle grey, partial conservation; light grey, low conservation). For more details see Table 1.

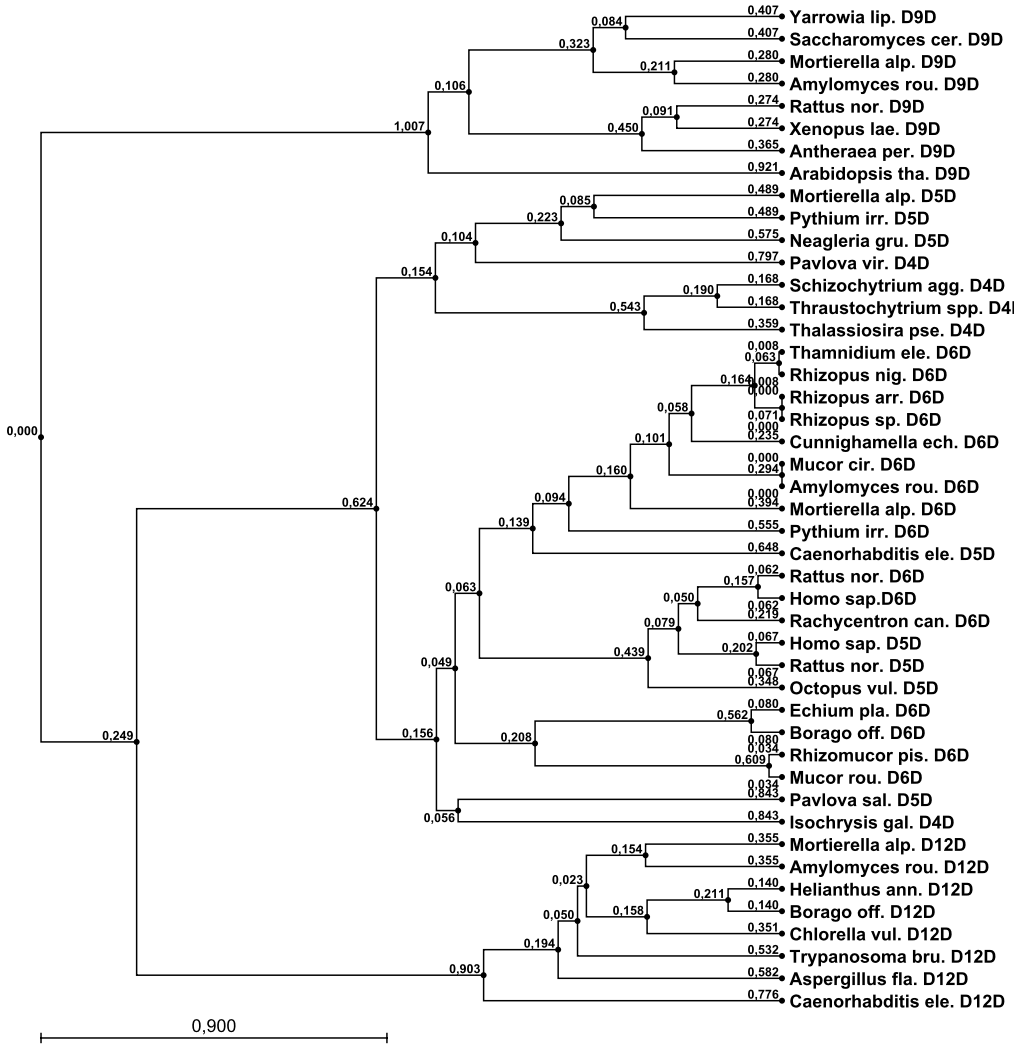

Figure 8. Phylogenetic tree of selected fatty acids desaturases. Phylogenetic tree was created by CLC Mainworkbech 5.1 (CLC Bio).

- The genes for membrane-bound desaturases can be divided into three big groups – Δ9 desaturases, Δ12 desaturases and "front-end" desaturases (Δ6, Δ5, Δ4 desaturases).
- Δ9 desaturases creates their own groups relatively distanced from the others. It could be assumed that these enzymes were the ancestor of all desaturases enzymes.
- The "front-end" desaturases cannot be separated into phylogenetic group based on the position specificity of enzyme, but they create small groups that are relative to taxonomy.
- It could be observed that fungal Δ6 desaturases from *Mucor rouxiii* and *Rhizomucor pissilus* are more close to plant Δ6 desaturases from *Borago* and *Echium* that to other fungal desaturases.

Physiological consequences of PUFAs on cell membranes

PUFAs are distributed to every cell membranes where are predominately esterified into the *sn*-2 position of phosphatidylcholine and phosphatidylethanolamine. PUFA effects on cell membrane functions have been noted and discussed widelyfrom the view of a) modifications of membrane fluidity, b) modifications of the activity of membrane bound enzymes, c) modifications of the number and affinity of receptors, d) modifications of the function of ion channels, and e) signal transduction (Yehuda et al. 2002; Hulbert et al. 2007; Abbott et al. 2012). Since the cell membranes are largely held together by van der Waals forces between fatty acyl chains, structural properties of these acyls (length and degree of unsaturation) play a key role in the membrane structure. The degree of unsaturation is particulary important because van

der Waals forces are acutely sensitive to the distance between interacting acyl chains. For example, whereas stearic acid is estimated to be 0.25 nm in diameter, oleic acid has spatial width of 0.72 nm (Cook 1991). The cis-unsaturated bond thus interrupts van der Waals forces between membrane fatty acids by increasing distance between them and lowers the membrane crystallization temperature. Therefore PUFA incorporation into membrane phospholipids would have an effect on the formation and stability of the membrane microdomains. As mentioned, the tight packing density of lipids in liquid ordered microdomains is conferred by favorable van der Waals forces between saturated acyl chains to hydrogen bonding between various membrane-associated molecules. PUFAs do not pack well with these molecules (e.g. sterols) and therefore form a liquid disordered phase (Edidin 2003). Fatty acid composition in the membrane lipids would also cause a redistribution of protein between liquid ordered and liquid disordered the membrane microdomains (Wickner 1977) as well as modify membrane-associated enzyme activity (Wilschut et al. 1978) and molecular transport (Tsong et al. 1977). In addition, PUFA acyl chains can rapidly convert between various conformational states (Shaikh and Edidin 2006). High conformational flexibility of both n-3 and n-6 PUFA acyl chains alters the physical properties of the membrane, including lateral organization, hydrophobic match, curvature stress, and the lateral pressure density profile, which can modify protein function, trafficking, vesicle budding, and fusion. Acyl chain flexibility also differs between n-3 and n-6 PUFAs, which may have functional consequences. This provides the strong evidence that composition and changes of membrane fatty acids play important role in the modification of cell membrane physiology (Watkins and German 2002).

Conclusion

The desaturation is the key reaction in biosynthesis of PUFAs. Characterization of genes and proteins of fatty acid desaturase enzymes is necessary step for their further applications. The field of possible usage is very wide including medicine, agriculture and food/feed industry. From the technological point of view, one of the most important applications is preparation of transgene plants with usage in oil industry. In December 2009, first plant enriched with gene for Δ6-desaturase was introduced to American market. SONOVA 400 is dicotyledon plant from clade *Asteridae*, *Carthamus tinctorius* (safflower), transformed with gene for Δ6- desaturase coming from fungi *Mortierella alpina* (Watkins 2010). Other technological possibilities are connected with preparation of highly oleaginous and highly specific microbial strains that could use low-cost agroindustrial wastes for effective conversion to either microbial oil with tailor-made PUFA composition or cereals enriched with PUFAs prepared by solid state fermentations. These are the possibilities of cost lowering that can help to spread PUFAs in human dietary. All of these efforts may demonstrate enormous potential for applications of fatty acid desaturases and thus create new perspectives market "bio-based" PUFA oils or products.

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