Genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans*

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Running title: *Aspergillus nidulans* delta-12 desaturase gene

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SUMMARY

In the Ascomycete fungus *Aspergillus nidulans*, the ratio of conidia (asexual spores) to ascospores (sexual spores) is affected by linoleic acid moieties including endogenous sporogenic factors called psi factors. Deletion of *odeA* (Δ*odeA*), encoding a delta-12 desaturase that converts oleic acid to linoleic acid, resulted in a strain depleted of polyunsaturated fatty acids (18:2 and 18:3) but increased in oleic acid (18:1) and total percent fatty acid content. Linoleic acid-derived psi factors were absent in this strain but oleic acid-derived psi factors were increased relative to wild type. The Δ*odeA* strain was reduced in conidial production and mycelial growth; these effects were most noticeable when cultures were grown at 26°C in the dark. Under these environmental conditions, the Δ*odeA* strain was delayed in ascospore production but produced more ascospores than wild type over time. This suggests a role for oleic acid-derived psi factors in affecting the asexual to sexual spore ratio in *A. nidulans*. Fatty acid composition and spore development were also affected by *veA*, a gene previously shown to control light driven conidial and ascospore development. Taken together our results indicate an interaction between *veA* and *odeA* alleles for fatty acid metabolism and spore development in *Aspergillus nidulans*.
INTRODUCTION

Several studies of filamentous fungi have suggested a role for 18:2 polyunsaturated fatty acids (i.e. linoleic acid) in fungal development, especially with regard to spore formation (1-4). In the filamentous fungus *Aspergillus nidulans*, linoleic acid-derived signal molecules called psi factors govern the development of cleistothecia (sexual bodies containing the sexual spores called ascospores) and conidiophores (asexual bodies producing the asexual spores called conidia) (5-8). Psi factor is a mixture of three hydroxylated linoleic molecules (PsiA1α, PsiB1α, and PsiC1α) and it has been reported that the proportion of these three compounds controls the ratio of asexual to sexual spore development in this fungus (5-8). Specifically, PsiB1α and PsiC1α are reported to stimulate sexual spore development whereas PsiA1α inhibits sexual spore development (6). Hydroxylated derivatives of oleic acid (PsiA1β, PsiB1β, and PsiC1β) have also been isolated from *A. nidulans* (7,8) but their role in sporulation has not been characterized.

In addition to the effects of psi factor on *Aspergillus* development, recent studies have also shown that purified linoleic acid and hydroperoxy linoleic acids derived from seed also exhibit sporogenic activities towards several *Aspergillus* spp. including *A. nidulans* and the seed infecting fungi *A. flavus* and *A. parasiticus* (9). In all of these species, the primary effect of linoleic acid and hydroperoxy linoleic acids was to induce precocious and increased conidial development. Lower concentrations of linoleic acid and 9(S)- hydroperoxy linoleic acid stimulated sexual spore development rather than conidial development in *A. nidulans* (9). These data suggest a relationship between linoleic acid and/or its derivatives and *Aspergillus* developmental processes.

The sporogenic effects of linoleic acid, hydroperoxy linoleic acids and psi factor were demonstrated on *A. nidulans* strains with an intact velvet (*veA*) locus (5,9). In *A. nidulans veA* strains, light delays and reduces sexual development and induces conidial production, while in
the absence of light conidial production is repressed and the fungus develops cleistothecia (10). Strains with mutations in *veA* (*veA1*) exhibit light-independent development of conidia and ascospores (10). Furthermore, *veA1* mutants do not develop spores in response to psi factor, linoleic acid and other linoleic acid derivatives (5,9). *veA* locus was originally identified by Käfer in 1965 (11), who also isolated the *veA1* mutation. *veA* has been sequenced (accession number U95045 and Yager personal communication). Currently the cellular function of VeA is not known.

As a first step in understanding the molecular genetics of psi factor formation and psi factor effects on *Aspergillus* development, we deleted the *odeA* gene encoding an oleate delta-12 desaturase, which catalyzes the conversion of oleic acid into linoleic acid. The effects of the *odeA* deletion were examined in both *veA* and *veA1* genetic backgrounds. The absence of *odeA* changed the fatty acid profile, including the composition of psi factor, and raised total percent fatty acids/fungal tissue. Interestingly, *veA* also affected fatty acid composition. Detailed examination of psi factor levels in the *veA* strains at 26°C indicated that, in comparison to the wild type, the ∆*odeA* strain was delayed in psi factor biosynthesis; however, higher amounts of psi factor were found over time. This was paralleled by delayed but increased ascospore production in this strain compared to wild type. The ∆*odeA* strain also displayed delayed and decreased conidial production compared to wild type in both *veA* and *veA1* backgrounds. Interactions between *odeA* and *veA* affected spore development, fatty acid composition and psi factor composition.
EXPERIMENTAL PROCEDURES

**Fungal Strains and Growth Conditions.** *Aspergillus nidulans* strains used in this study are listed in Table 1. Cultures were grown on *A. nidulans* glucose minimal medium (GMM) unless otherwise indicated. GMM consists of 10 g glucose, 6 g NaNO₃, 0.52 g KCl, 0.52 g MgSO₄·7H₂O, 1.52 g KH₂PO₄, 1 ml trace elements (2.2 g ZnSO₄·7H₂O, 1.1 g H₃BO₃, 0.5 g MnCl₂·4H₂O, 0.5 g FeSO₄·7H₂O, 0.16 g CoCl₂·5H₂O, 0.16 g CuSO₄·5H₂O, (NH₄)₆Mo₇O₂₄·4H₂O, 5 g Na₄EDTA in 100 ml distilled H₂O), in 1L distilled H₂O. pH was adjusted to 6.5 with a 10N NaOH solution. Appropriate supplements corresponding to the auxotrophic markers were added to the medium (12). Agar (15 g/L) was added to obtain solid medium. Temperature of incubation was 37°C, unless indicated otherwise. Cultures were grown in continuous white light or in the dark. Cultures requiring white light were grown in an incubator equipped with General Electric 15-W broad-spectrum fluorescent light bulbs (F15T12CW) positioned at a distance of 20 cm from the agar surface, with a light intensity of 66 mE/m²/s.

**Identification and cloning of the *A. nidulans* delta-12 desaturase gene.** An *A. nidulans* cosmid library (pWE15, Fungal Genetics Stock Center, Kansas City, Kansas) and *A. parasiticus* cosmid library (pA1, provided by J. Linz, 13) were screened using a putative delta-12 desaturase gene from the yeast *Candida albicans*. The gene was contained in a 1.8 Kb EcoRI fragment obtained from the plasmid pFAD4 (provided by J. Beckerman and P. MaGee). The screening of the *A. parasiticus* library yielded a cosmid, pAMC8, containing the putative delta-12 desaturase gene. A 1.2 Kb BamHI fragment from pAMC8 containing the carboxy terminal coding region of the *A. parasiticus* delta-12 desaturase gene was used as a probe to screen the *A. nidulans* pWE15 genomic cosmid library, and a single cosmid, pWEO2H5, was obtained. Fragments from these cosmids were subcloned to facilitate sequencing and construction of transformation vectors.
**Sequence analysis.** Fragments from the cosmid pWEO2H5 were subcloned into the plasmid pK19 (14), obtaining pAMC15 (4.6 Kb *Hind*III insert), pAMC16 (2.4 Kb *Kpn*I insert), pAMC17 (6 Kb *Sal*I insert), pAMC18 (1.8 Kb *Bam*HI insert) and pAMC19 (1.3 Kb *Bam*HI/*Hind*III insert). DNA sequencing of both strands was performed using synthetic primers and ABI PRISM DNA Sequencing kit (Perkin-Elmer). Sequences were assembled with the Sequencher 3.1 program. Nucleotide sequence was translated in all six reading frames using BLASTX 2.0.12 and compared with the sequences in Genbank (15). The Genbank sequence accession number of *A. nidulans odeA* is AF262955.

**Deletion of A. nidulans odeA.** The transformation vector utilized to delete *odeA* was called pAMC31.3. This plasmid included the *argB* marker gene and *odeA* flanking sequences without the *odeA* encoding region. pAMC31.3 was constructed as follows: first, the plasmid pAMC26.6 was obtained by insertion of a 7 Kb *Sph*I-*Xho*I fragment from pWEO2H5 into the *Sph*I-*Sal*I sites in pK19 (14). Another plasmid, pAMC29.1, was generated by digestion of pBlueScript SK- (Stratagene) with *Eco*RI and *Xho*I, followed by a blunt-end reaction and religation of the plasmid. A 4.3 Kb *Sma*I fragment, containing the 1.3 Kb *odeA* encoding region, was released from pAMC26.6 and ligated into the *Sma*I site in pAMC29.1 to create pAMC30.4. The entire *odeA* encoding region plus 389 bp downstream from the putative stop codon was then removed from pAMC30.4 by a *Sal*I-*Nde*I double digest. This left two 1.3 Kb genomic DNA fragments that were on either side of the excised *odeA* gene. The remaining linear vector was blunt-ended and ligated to a blunt-ended 1.8 Kb fragment containing the *A. nidulans argB* gene to obtain the final transformation vector pAMC31.3. Using standard procedures (16), *Aspergillus nidulans FGSC89 (biA1; argB2)* (Table 1) was transformed with pAMC31.3 to create TAMC31.65 (*biA1; veA1, ΔodeA*). Replacement of *odeA* by *argB* is denoted by the symbol Δ*odeA*. The Δ*odeA* allele was introduced in a veA background by sexual recombination of TAMC31.65 with
WIM126 (pabaA1; veA). Ultimately, four isogenic strains differing only in veA and odeA alleles were created through transformation and sexual crosses (Table 1).

Complementation of odeA deletion strain. The transformation vector pAMC33.3 was used to complement the odeA deletion strain RAMC25 (pyroA4; veA1, ΔodeA). pAMC33.3 was constructed by inserting the 4.3 Kb SmaI fragment from pAMC26.6 into pSM3 (which contains A. nidulans pyroA as a selectable marker, 17).

Southern analysis. Approximately 5 µg of genomic DNA from transformed A. nidulans strains were double digested with Sall/NdeI, and another 5 µg were digested with BamHI. Genomic DNA samples were separated by electrophoresis in a 1% agarose gel and then transferred to Hybond membrane (Amersham) by capillary action. Gene replacement of odeA with argB was confirmed by probing with a 1.7 Kb Sall-NdeI fragment from pAMC30.4 and a 4.3 Kb SmaI fragment from pAMC26.6. DNA was labeled with P32 by random primer extension as described by Sambrook (18).

mRNA studies. Total RNA was isolated from mycelia by using Trizol as described by the supplier (Gibco BRL). Approximately 20 µg of total RNA were used for RNA blot analysis. Temperature and light effects on expression of odeA and the A. nidulans delta-9 desaturase gene (sdeA) were analyzed by using A. nidulans veA1; veA1, ΔodeA; veA; and veA, ΔodeA strains. Inocula (10^6 conidia/ml) were grown in 3 ml of liquid GMM in 7 ml glass vials under stationary conditions for 72 h. Cultures were grown at 20 °C, 26 °C, 30 °C, 37 °C and 40 °C in the dark. Light effects were observed in cultures grown at 37 °C. The 1.7 Kb Sall-NdeI fragment containing the odeA encoding region and a sdeA cDNA fragment (an EST clone encoding the putative delta-9 desaturase of A. nidulans, provided by Drs. B. Roe and D. Kupfer) were used as probes.
Fatty acid feeding studies were carried out in the four near-isogenic strains (i.e. veA1; veA1, ΔodeA; veA; and veA, ΔodeA). Inocula (10^6 conidia/ml) were grown in 1 L flasks containing 400 ml of liquid GMM at 37 °C and 300 rpm. After 16 h of growth, equal amounts of mycelia were transferred into GMM plus sodium linoleate, GMM plus sodium oleate, GMM, control minimum medium without glucose (MM) plus sodium linoleate, MM plus sodium oleate and MM. Sodium linoleate and sodium oleate (Sigma) were added to a final concentration of 1 mM in 1% tergitol NP-40 (19). Controls containing tergitol, GMM plus tergitol and MM plus tergitol, were also included in the experiment. After 8 h the mycelia were harvested and processed for mRNA analysis.

Physiological studies. Conidial production studies were performed on plates containing GMM agar plus appropriate supplements that were spread with 100 µl of water containing 10^5 conidia of veA1; veA1, ΔodeA; veA; and veA, ΔodeA strains. The cultures were incubated in the dark at 26° C and 37° C, and in the light at 37° C. After 72 h, a core of 12.5 mm diameter was removed from each plate and homogenized in 2 ml of water to release the spores. Conidia were counted using a hemacytometer. Colony growth was recorded as colony diameter. The experiments were carried out in triplicate.

The studies on sexual spore production were performed with the veA and veA, ΔodeA strains. The strains were inoculated on YGT medium, since this medium has been used in previous research to promote sexual development in A. nidulans (5,6,9). Five ml of melted 0.7% agar-YGT containing 10^6 conidia were poured on 30 ml of solid 1.5% agar-YGT and incubated in the dark at 26° C and 37° C, and in the light at 37° C. After 10 days, a core of 12.5 mm diameter was removed from each plate and homogenized in 2 ml of water to release the spores. Ascospores were counted using a hemacytometer. The experiments were performed with four replicates. Additionally, in order to study the early stages of sexual development in the absence of odeA, a time course was carried out taking microscopic observations at 42 h, 66 h, 90 h, 114 h,
138 h and 162 h after inoculation. Free ascospores and mature asci of veA and veA, ΔodeA strains were counted at the 162 h time point following the procedure described above.

Fatty acid methyl esters (FAME) analysis. veA1; veA1, ΔodeA; veA; and veA, ΔodeA strains were incubated in 15 ml liquid GMM in 8 cm diameter plates under stationary conditions at 37°C in the light, 37°C in the dark or 26°C in the dark. After 72 h, the mycelial mats were harvested and lyophilized to analyze FAME composition, including psi factors. Samples from veA and veA, ΔodeA on YGT medium were also analyzed at 42 h, 66 h, 114 h, 162 h, and 240 h for psi factor and FAME composition. Mycelia (56 to 134 mg) were sequentially extracted with two portions of 15 ml chloroform/methanol (2:1, v/v) at –20°C for a total of 4 d followed by extraction with 20 ml of ethyl acetate/methanol (1:1, v/v) for 4 h at room temperature with agitation. A known quantity of methyl nonadecanoate (19:0, NuChek Prep) was added to the combined extract of fungal lipids (19:0 was previously shown to be absent in Aspergillus strains), and the extract was evaporated to dryness. The lipid residue was partitioned with 20 ml chloroform/methanol/water (2:1:1, v/v/v), and the chloroform layer was collected, and evaporated. The resultant lipid residue was saponified with 2 ml 0.5 N NaOH in methanol at 65°C for 30 min, after which the solution was acidified with 1 M oxalic acid. Free fatty acids extractable with chloroform after partition in chloroform/methanol/water (2:1:1, v/v/v) were methyl esterified with an excess of diazomethane in diethyl ether/methanol (9:1, v/v) for 2 min at room temperature. FAME recovered after solvent removal were partitioned with 6 ml hexane/methanol (2:1, v/v), and 20 μg of internal standard ethyl ricinoleate (Sigma) was added for determination of hydroxy fatty acid methyl esters (HFAME). FAME were more selectively concentrated in the hexane layer, while HFAME were mainly extracted into the methanol layer. FAME, including the 19:0 internal standard, were analyzed by flame ionization detection-gas chromatography (FID-GC) by removing samples from the hexane layer. FID-GC was completed with a Spectra Physics Model SP-7100 gas chromatograph equipped with a flame ionization detector and a capillary column (0.25 mm X 25 m; film thickness, 0.25 mm; coated with a film
of 007 CPS-2, J and S Scientific). The carrier flow was 1 ml/min, and the temperature programming was 100°C to 180°C at a rate of 3°C/min.

After FAME analysis, the methanol layer from the hexane/methanol partition, containing HFAME, was recovered, and evaporated to dryness. The residue was treated with trimethylchlorosilane/hexamethyldisilazane/pyridine (3:2:2, v/v/v) to obtain trimethylsilyloxy (OTMSi) derivatives of the hydroxyl groups. After 15 min, the reagent was evaporated and 100 µl hexane was added for analyses by FID-GC. OTMSi HFAME were analyzed with a Hewlett-Packard Model 5890 gas chromatograph equipped with flame ionization detection using the internal standard OTMSi ethyl ricinoleate. The capillary column used was a SPB-1 (dimethyl polysiloxane phase, 0.32 mm X 30 m, film thickness 0.25 µm, Supelco). Temperature programming was from 160°C to 260°C at 5°C/min with a hold at 260°C for 5 min; the flow was 2 ml/min.

The identity of the FID-GC peaks were confirmed by GC-MS using a Hewlett-Packard Model 5890 gas chromatograph interfaced with a model 5971 mass-selective detector operating at 70 ev. The capillary column utilized was a Hewlett-Packard HP-5MS cross-linked 5% phenyl methyl silicone (0.25 mm X 30 m, film thickness 0.25 µm). FAME were analyzed by temperature programming from 140°C to 260°C at a rate of 5°C/min with a flow rate of 0.67 ml/min. HFAME were analyzed identically, except the temperature was programmed from 160°C to 260°C at a rate of 5°C/min with a hold at 260°C for 10 min.

The identity of methyl 8-hydroxy-9(Z)-octadecenoate (8-HOE), psiB1β, as its OTMSi ether was also confirmed by its isolation by thin-layer chromatography (TLC) followed by analyses by both GC-MS and proton nuclear magnetic resonance (1H-NMR). 1H-NMR was completed with a Bruker model ARX-400 spectrometer (400 MHz) using C2HCl3 as an internal standard and solvent. Lipid (as methyl ester/OTMSi ether derivative) from the ∆odeA strains
was separated by TLC (Silica Gel 60 F254 plates, 20 cm X 20 cm X 0.25 mm, Merck) using solvent development with hexane/ethyl ether (9:1, v/v). After spraying the plate with 0.1% aqueous sodium 8-anilino-1-napthalenesulfonate, 8-HOE (methyl ester/OTMSi ether) was detected at $R_f = 0.41$ by long-wave ultraviolet light. The scrapings containing 8-HOE (methyl ester/OTMSi ether) were eluted with ethyl acetate, and the solvent was removed for analysis.

**Statistical analysis.** Chemical and sporulation data were evaluated by analysis of variance (ANOVA). To compensate for the fact that the experimental design was not fully factorial (i.e. all possible combinations of temperature and illumination were not tested) each condition ($26^\circ$C dark, $37^\circ$C dark, $37^\circ$C light) was treated as an independent category within a single independent variable "environmental treatment". For cases in which the main effect of environmental treatment was significant, single degree of freedom contrasts to test the specific hypotheses of the effect of temperature ($37^\circ$C dark versus $26^\circ$C dark) and the effect of illumination ($37^\circ$C dark versus $37^\circ$C light) were performed.
RESULTS

Sequence analysis of odeA and complementation of the ΔodeA deletion with a wild type odeA gene.

Sequence analysis of *A. nidulans* *odeA* at both the nucleotide and amino acid level revealed high similarity with other delta-12 desaturases from plants and fungi. OdeA contained the conserved His-rich regions found in other delta-12 desaturases (data not shown) (20-22). Deletion of *odeA* resulted in loss of polyunsaturated fatty acid biosynthesis and alterations in spore production (described below). Transformation of the Δ*odeA* strain with the *odeA* gene recovered the wild type phenotype as revealed by fatty acid analysis and physiological studies (data not shown). This supports the conclusion that the defects in the Δ*odeA* phenotype are solely due to loss of the *odeA* gene.

Effect of *odeA*, veA and environment on *A. nidulans* fatty acid composition.

Due to the extensive use of *veA1* strains as research models throughout the international research community, the *odeA* deletion was placed in both a *veA* and *veA1* genetic background, although our main interest was examining sporulation and psi factor composition in the *veA* strains. Also, because fatty acid composition has been shown to change with temperature (23,24), fatty acid composition was compared in the *veA; veA1; veA, ΔodeA;* and *veA1, ΔodeA* strains at both 26°C and 37°C. Fatty acids were also examined under light and dark regimes at 37°C due to the importance of light in conidial versus ascospore development in *veA* strains (10).

Fatty acid composition was first examined under culture conditions known to promote asexual spore development (72 h growth and GMM medium). Table 2 shows the fatty acid composition of *veA; veA1; veA, ΔodeA;* and *veA1, ΔodeA* under the environmental conditions (treatments) tested. *OdeA* allele, *veA* allele and treatment had a significant effect on fatty acid
composition (P < 0.01). There was a near loss of polyunsaturated fatty acids (PUFA, 18:2 and 18:3) in ΔodeA strains (Table 2). Their presence was however confirmed independently by silver-ion HPLC followed by GC-MS of the HPLC-purified fame (not shown). To determine whether these low levels of PUFA were of fungal or exogenous origin in the ΔodeA strains, GMM medium was examined for the presence of linoleic acid. The medium contained trace amounts of linoleic acid (0.8 µg), but this amount was negligible in comparison to the total amount found in ΔodeA mycelia (average of 23 µg per culture). Levels of other fatty acids also changed in ΔodeA mycelia (Table 2). For example, the percentage of saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0) was reduced. In addition, the total percentage of FAME per g mycelium was approximately two to three-fold higher in the ΔodeA strains. These effects were conserved independently of veA or veA1 alleles, illumination regimens and temperature (Table 2).

The veA allele also had a significant effect on the fatty acid profile (P < 0.01). The veA strain contained less linoleic acid and linolenic acid but more oleic and stearic acid than veA1 (Table 2). An interaction between veA and odeA alleles on the relative percent of each fatty acid except stearic acid was illustrated by the fact that variations in these fatty acids between veA and veA1 strains were not maintained in the ΔodeA background (P < 0.01, Table 2).

Treatment (temperature and light) also significantly (P < 0.01) affected fatty acid composition. Linolenic acid (18:3) percentage increased at 26°C, regardless of veA alleles. At this temperature, stearic acid (18:0) percentages also increased and palmitic acid (16:0) decreased, regardless of veA or odeA alleles. An interaction (P < 0.01) between environmental treatment and odeA was observed in the decrease in linoleic acid at 26°C in wild type odeA but not ΔodeA strains. This was expected, as the ΔodeA strain produced virtually no linoleic acid.

Identification of the psi factors
Psi factors from *A. nidulans*, 8-hydroxy-9(Z),12(Z)-octadecadienoic acid (8-ODE = PsiB1\(\alpha\)), 8-hydroxy-9(Z)-octadecenoic acid (8-HOE = PsiB1\(\beta\)), 5,8-dihydroxy-9(Z),12(Z)-octadecadienoic acid (5,8-diHODE = PsiC1\(\alpha\)), and 5,8-dihydroxy-9(Z)-octadecenoic acid (5,8-diHOE = PsiC1\(\beta\)) were previously identified by Mazur et al. (8). We examined these HFAME as methyl ester/OTMSi ether derivatives whereas Mazur et al. (8) reported mass spectra of the methyl esters. The mass spectra of 8-ODE and 8-hydroxy-9,12,15-octadecatrienoic acid (as methyl esters/OTMSi ethers) were similar to those reported previously by Brodowsky and Oliw (25). The latter HFAME, derived from 18:3, was found only in cultures incubated at 26°C; furthermore, it was a minor component compared to the other HFAME (data not shown). This compound has not been previously identified in *A. nidulans* and we give it the term PsiB1\(\gamma\). No PsiA1 was detected in any samples.

8-HOE (methyl ester/OTMSi ether) was isolated from TLC and examined by \(^1\)H-NMR and GC-MS. The \(^1\)H-NMR spectrum was consistent with the structure of 8-HOE as follows in chemical shift (\(\delta\)), multiplicity (singlet, s; doublet, d; triplet, t; multiplet, m), and coupling constant (\(J\)): TMSi, 0.15 \(\delta\), s; C-18, 0.87 \(\delta\), t; C-4 to C-7 and C-12 to C-17, 1.24 to 1.31 \(\delta\), m; C-4, obscured by water impurity; C-11, 2.03 \(\delta\), m; C-2, 2.29 \(\delta\), t; ester methyl, 3.65 \(\delta\), s; C-8, 4.41 \(\delta\), dt; C-9, 5.34 \(\delta\), ddt, \(J_{9,10} = 10.8\), \(J_{8,9} = 8.9\), \(J_{9,11} = 1.4\); C-10, 5.47 \(\delta\), dt, \(J_{9,10} = 10.8\), \(J_{10,11} = 7.4\). The electron-impact mass-spectra (EI-MS) of 8-HOE (methyl ester/OTMSi ether) was as follows in \(m/z\), [ion structure], and (relative intensity): 384 [M]+ (0.2), 369 [M – CH\(_3\)]+ (1), 353 [M – OCH\(_3\)]+ (0.3), 337 [M – CH\(_3\) – HOCH\(_3\)]+ (1), 294 [M – TMSiOH]+ (0.3), 271 [M – (CH\(_2\))\(_2\)CH\(_3\)]+ (2), 241 [M – (CH\(_2\))\(_6\)COOCH\(_3\)]+ (100), 216 (2), 159 (2), 155 (2), 143 (4), 129 (51), 94 (15), 73 [TMSi]+ (59).

The EI-MS of the other HFAME (methyl ester/OTMSi ether) were as follows in \(m/z\), [ion structure], and (relative intensity): 5.8-diHODE; 349 [M – TMSiOH – OCH\(_3\)]+ (0.4), 290 [M – 2 TMSiOH]+ (6), 282 (5), 269 (7), 239 [M – (CH\(_2\))\(_2\)CHOTMSi(CH\(_2\))\(_3\)COOCH\(_3\)]+ (19), 216 (4),
Effect of odeA, veA and environment on A. nidulans psi factor composition in GMM medium.

Psi factor composition was examined in 72 h GMM cultures (Table 3). Under these conditions, odeA allele, veA allele and treatment had significant effects (P < 0.01) on psi factor composition. odeA deletion resulted in the loss of detectable psiB1α. PsiB1β levels were greatly increased in the ΔodeA strains except in the veA, ΔodeA strain at 26°C. An interaction between odeA and veA was also apparent by the high amount of psiC1β found only in the veA1, ΔodeA strain (Table 3).

Other observations included detection of 10-hydroxy-8,12-octadecadienoic acid in veA1 and veA strains and 10-hydroxy-8-octadecenoic acid in all the strains (data not shown). This is the first report of their presence in A. nidulans although these PUFA have been detected in other fungi (25-27). Currently, it is not known if they also act as sporogenic elements.

Fatty acid and psi factor composition during sexual stage development is affected by odeA.

Because sexual development has typically been assessed by growth of veA strains on YGT medium and we are attempting to examine a possible role of psi factor composition in sexual development, we looked at psi factor and fatty acid composition in veA strains (wild type odeA and ΔodeA backgrounds) grown on this medium. Both psi factor analysis and microscopic observations were performed at the same time points for these strains.
Figure 1 shows the changes in relative percent of FAME composition over time in veA (Fig. 1A) and the veA, ΔodeA strains (Fig. 1B). In both strains, the most unsaturated fatty acid (linoleic in veA and oleic in veA, ΔodeA) showed a similar trend of decreasing in percentage composition at 66 and 114 h but increasing in percentage at 162 h and 240 h. The percent composition of the other detectable fatty acids showed an opposite pattern in both strains.

The FAME weight/mycelium weight peaked at 66 h in both the veA strain and the veA, ΔodeA strain. As major components of the total FAME, both linoleic acid and oleic acid reflected the trend of the total FAME weight/mycelium weight (Fig. 1C). On a weight basis/g of mycelium, there was a correlation in linoleic acid versus PsiB1α plus PsiC1α, as well as a correlation in oleic acid versus PsiB1β plus PsiC1β. It was also noteworthy that compared to linoleic acid, oleic acid appeared to yield a much greater quantity of psi factors. This greater accumulation of oleic acid-derived psi factor proved to be valid when all values obtained in this study were examined. Linear plots of oleic acid versus PsiB1β plus PsiC1β (r = 0.873) and linoleic acid versus PsiB1α plus PsiC1α (r = 0.715) showed that oleic acid-derived psi factor accumulated 2.5-fold greater per quantity of oleic acid compared to linoleic acid-derived psi factor from linoleic acid. In general, about 5 to 5.8 µg PsiB1β plus PsiC1β were found per mg oleic acid, and about 2.2 µg PsiB1α plus PsiC1α accumulated per mg linoleic acid. Our examination also showed that although psi factor accumulation was delayed in the veA, ΔodeA strain, more psi factor was found in this strain at time points later than 42 h (Fig. 1D).

Effect of odeA, veA and environment on A. nidulans developmental processes.

Vegetative growth and asexual spore production were assessed in both veA and veA1 backgrounds. However, sexual development was assessed solely in veA strains because the veA allele is required for response to psi factor, and because sexual development has been better characterized in veA strains (5-10).
I. Vegetative growth. Figure 2A shows that environmental treatment, veA allele and odeA allele had a significant effect (P < 0.01) on A. nidulans colony diameter. In wild type odeA backgrounds, the veA strain had a larger colony diameter than the veA1 strain. This was not true in ΔodeA backgrounds, demonstrating an interaction between veA and odeA alleles (P < 0.01). The lack of the odeA allele led to significant reduction in colony diameter. These effects were maintained over 5 days of incubation (data not shown).

II. Asexual spore development. Environmental treatment, veA allele and odeA allele had a significant effect on conidial production (P < 0.01; Fig. 2B). There were also significant interactions between veA and odeA, veA and environmental treatment, and odeA and environmental treatment (P < 0.01). There was a significant decrease in conidial production in the veA1, ΔodeA and veA, ΔodeA strains at 26°C (Fig. 2B). However, at 37°C only the veA1, ΔodeA strain showed a reduction in conidial production in comparison to the wild type. Both veA and veA, ΔodeA strains exhibited the light/dark response characteristic of a veA wild type allele which was to produce more conidia in the light than in the dark. No significant differences in conidial production were observed in veA1 strains, wild type odeA or ΔodeA, with respect to illumination conditions (Fig. 2B). Lower temperature decreased conidial production in all veA1 strains, but only in the veA strain containing the ΔodeA allele.

III. Sexual spore development. Our studies indicated that light and temperature and the odeA interaction with light and temperature significantly affected ascospore development in A. nidulans (Fig. 3A and data not shown). As observed before (5,9,10), the wild type veA strain produced more ascospores in the dark than in the light at 37°C (P < 0.01). This effect was also observed in the ΔodeA background (P < 0.05). The ΔodeA strain showed an increase in ascospore production relative to the wild type odeA strain in 10-day old cultures grown at 26°C.
(Fig. 3A, P < 0.01), but no significant differences between these strains were observed at 37°C in light or dark grown cultures.

To further investigate the effect of the odeA deletion on sexual development at 26°C, we made microscopic observations of sexual development from 42 h to 162 h on the wild type and ΔodeA strain. At 42 h, only hyphal growth was present in both cultures. Hülle cells were observed at 66 h and 90 h in both strains. At 114 h and 138 h, the cleistothecial walls were forming. At 162 h ascus (at different stages of maturity) and free ascospores were present. Quantitative analysis showed that the numbers of mature ascus and free ascospores were higher in the wild type than in the ΔodeA strain at 162 h (Fig. 3B). This was in contrast to the ΔodeA 10-day old culture, which showed an increase in ascospore production with respect to the wild type odeA strain (as mentioned above, Fig. 3A).

odeA and sdeA are temperature and light regulated.

Positive regulation of desaturase genes by low temperatures and light has been reported in other organisms (24,28-32). We studied the effect of temperature and light on odeA and sdeA expression in A. nidulans. As previously mentioned, sdeA encodes a putative delta-9 desaturase in A. nidulans, that is responsible for the conversion of stearic acid into oleic acid. Figure 4 shows that both odeA and sdeA transcript accumulation was induced by low temperatures (26°C and 20°C) in both veA and veAl strains and by light (only examined at 37°C) in veAl strains. As expected, no odeA transcripts were observed in the ΔodeA strains (Fig 4B and 4D). sdeA transcripts were elevated in the ΔodeA strains (Fig. 4).

Polyunsaturated fatty acid-regulated expression of odeA and sdeA.

The increase in sdeA transcript in the ΔodeA strains suggested a possible regulation of PUFA on sdeA expression. To further investigate this possibility, all four strains were grown in various carbon sources including unsaturated fatty acids. In the odeA wild type strains, odeA and
sdeA expression was notably higher in the presence of glucose than in its absence, however this was not observed in ΔodeA strains (Fig. 5B and 5D). When the ΔodeA strains were grown in linoleic acid as a sole carbon source there was a notable decrease in sdeA transcript accumulation in both veA and veA1 strains. This decrease was attenuated by the addition of glucose in the medium (Fig. 5B and 5D). mRNA analysis also showed that the accumulation of odeA transcripts was higher in the veA strain than in veA1 strain (Fig. 5A and 5C). In the veA strain, a slight reduction in odeA and sdeA transcript accumulation was also observed when exogenous linoleic acid was added (Fig. 5A).
DISCUSSION

The genus *Aspergillus* contains many industrially, medically and agriculturally important species whose mode of reproduction depends primarily on the production of asexual spores called conidia and, for some species, sexual spores called ascospores. Factors contributing to spore development of this genus include linoleic acid (9) and various oxidized derivatives of linoleic acid. These include endogenous *A. nidulans* sporogenic molecules called psi factor (5-8) and plant defense metabolites, 9(S)- and 13(S)- hydroperoxy linoleic acid (9). This latter point is of significance as many *Aspergillus* spp. are seed infesting fungi that elicit hydroperoxy linoleic acid production in higher plants (33). The sporogenic response to linoleic acid moieties requires the presence of an intact *veA* gene. However, as *veA1* mutant strains have been historically used by the research community due to their convenient trait of developing asexually in the dark, we have investigated the role of linoleic acid and psi factor on fungal development through characterization of both *A. nidulans veA* and *veA1* strains deficient in linoleic acid biosynthesis.

As expected, chemical analysis of ∆odeA strains demonstrated the absolute requirement of OdeA for normal fatty acid metabolism in *A. nidulans* (Table 2 and Fig. 1A and 1B). In contrast to odeA strains, where linoleic acid content was ~ 50% of FAME, the ∆odeA strains presented only trace amounts of linoleic acid. The odeA deletion also resulted in a 2-3 fold increase in total fatty acids/weight of fungal biomass. Moreover, the chemical makeup of the fatty acid profile was altered in these strains: palmitic acid content was decreased and both stearic and oleic acid content increased compared to wild type strains. The extraordinarily high amount of oleic acid was likely due not only to the block in the fatty acid pathway, but also to the increase in *sdeA* transcript accumulation in the ∆odeA strains (Fig. 4 and 5).
We also found that fatty acid composition was affected by \textit{veA}. The differences observed between \textit{veA} and \textit{veA1} strains with respect to the fatty acid profile were medium-dependent. Lower amounts of PUFA and higher amounts of monounsaturated fatty acids were found in \textit{veA} strains compared to those found in \textit{veA1} strains in glucose minimum medium, a medium which promotes asexual spore development (Table 2). The inverse was observed when the fungal strains were grown in YGT (data not shown), the medium used for promoting the sexual stage in \textit{A. nidulans} (5-8). Furthermore, there were significant interactions between \textit{veA} and \textit{odeA} alleles on fatty acid metabolism as detailed in the result section (Tables 2 and 3; Fig. 4 and 5). This suggests a complex regulation of fatty acid metabolism involving \textit{veA} and fatty acid biosynthetic genes.

Elimination of \textit{odeA} also led to changes in both \% psi factor/weight of fungal biomass and psi factor composition. Both PsiB1\(\alpha\) and PsiB1\(\beta\) were found in the wild type strain, but the \(\Delta\)\textit{odeA} strain was crippled in its ability to synthesize PsiB1\(\alpha\) (Table 3). Instead, high levels of PsiB1\(\beta\) and PsiC1\(\beta\) were found in the mutant strain. Furthermore, an interaction between \textit{veA} and \textit{odeA} alleles was demonstrated by the fact that the PsiB1\(\beta\) and PsiC1\(\beta\) levels were statistically greater in the \textit{veA1}, \(\Delta\)\textit{odeA} strain versus the \textit{veA}, \(\Delta\)\textit{odeA} strain (Table 3). Also, when grown at 26\(^{\circ}\)C in YGT medium, psi factor was not detected in the \(\Delta\)\textit{odeA} strain until 66 h, at which time the level of psi factor was 5-fold above that of wild type (Fig. 1D). Although there were differences in the amount of psi factor found dependent on experiment (Fig. 1D, Table 3), in general the total amount of psi factor detected in \(\Delta\)\textit{odeA} strains was several fold greater than that of wild type \textit{odeA} strains.

Experiments by Champe et al. (5,6) led to the hypothesis that PsiB1\(\alpha\) and PsiC1\(\alpha\) play a prominent role in increasing the sexual to asexual spore ratio in \textit{A. nidulans} but no experiments were conducted with PsiB1\(\beta\) and PsiC1\(\beta\) to determine if they also had a role in spore development. Although we did not directly assess the effect of PsiB1\(\beta\) or PsiC1\(\beta\) on \textit{Aspergillus}...
development, our results suggest that these derivatives may also act as sexual sporogenic factors as the increase in ascospore numbers in the $\Delta odeA, veA$ strain at 240 h and 26°C (Fig. 1B and 3A) was accompanied by an increase in $\Psi_2$ level. Our data also suggested that the oleic acid:linoleic acid ratio may be playing a role in the relative development of conidia and ascospores. We note that in *Neurospora crassa* oleic acid is the predominant fatty acid found in developing asci and mature ascospores, whereas linoleic acid is the predominant fatty acid in asexual tissue in this fungus (34).

The $\Delta odeA$ strains produced less conidia than the $odeA$ wild type strains, especially at low temperatures (Fig. 2B). Aside from some possible role of the oleic acid:linoleic acid ratio on directing asexual to sexual spore development, this decrease could also be explained as a need for high PUFA content for conidial formation in cold environments. Temperature had a decided effect on $odeA$ and $sdeA$ transcript accumulation; both were more abundant when the fungus was grown at lower temperatures (Fig. 4). The adaptation of cells to maintain the membrane fluidity in response to a downward shift in temperature by desaturating fatty acids has been studied in higher plants (35-37), animals (38) and in cyanobacteria (39-41). Low-temperature induction of desaturase genes has been reported in cyanobacterium species (24,30,31). Positive regulation of a fungal delta-9 desaturase gene by low temperature has been described previously in fungi in *Mucor rouxii* (32). Considering the increase in linolenic acid (18:3) in cultures grown at 26°C (Table 2), it is also likely that *A. nidulans* contains an omega-3 desaturase positively regulated by low temperature in a similar manner as described in cyanobacteria (24,30,31).

A most interesting observation in this study was the response of the $odeA$ and $sdeA$ alleles to light. There was light induction of $odeA$ and $sdeA$ transcription but only in the strains containing the $veA1$ allele. These results also indicate another possible genetic link between $veA$ and $odeA$. Perhaps this response is part of the reason that there are differences seen in the fatty acid profile between $veA$ and $veA1$ strains. Light-induced transcription of green algae and plant
disperses have been recorded (28,29) but this is the first report of a fungal disperses that responds to light.

The increased expression of the sdeA gene in the ΔodeA strain (Fig. 4 and 5) and the high levels of oleic acid in the ΔodeA strain suggest a role of OdeA and/or linoleic acid in regulating fatty acid desaturation in A. nidulans. Additionally, we found that exogenous linoleic acid partially repressed sdeA expression (Fig. 5A, 5B, 5D). Feedback regulation of delta-9 desaturase activity has also been noted in mammals (42-45) and yeast (19). The attenuation of the negative regulation of sdeA transcript in ΔodeA strains when grown in medium containing glucose indicates interactions between carbon metabolism and PUFA metabolism. PUFA have also been shown to negatively regulate fatty acid synthase, the first committed step in fatty acid metabolism, in mammals (46). We suggest that depletion of PUFA in the ΔodeA strain derepresses PUFA regulation of fatty acid metabolic genes leading to the observed three-fold increase in total fatty acid content of this strain.

In conclusion, we have shown that fatty acid composition in A. nidulans varies during spore development and is influenced by odeA, veA, temperature and light. Both odeA and veA alleles are required for normal asexual and sexual spore development. Although fatty acid and psi factor composition alters with mutations in both of these alleles, it is not yet possible to attribute asexual or sexual spore production to presence of specific fatty acids as other aspects of fungal physiology also changed. We hope by characterizing sdeA mutants and genes involved in psi factor formation to further elucidate the role of oleic acid or oleic acid psi factors in spore development. We also found that odeA and sdeA expression, like that of other desaturase genes, is responsive to environmental factors including temperature and light and that OdeA and/or linoleic acid play a role in regulating fatty acid desaturation. In addition, it is important to note that even though most of the Aspergillus research community investigates on veA1 strains, the
veA1 mutation leads to major changes in sporulation, fatty acid and psi factor profiles. These results open the possibility that previous findings in veA1 might not always apply in veA strains.
ACKNOWLEDGMENTS

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REFERENCES


FIGURE LEGENDS

Fig. 1. FAME composition in veA (A) and veA, ΔodeA (B) strains; FAME weight per mycelium weight (C) and psi factor weight per mycelium weight (D) of veA and veA, ΔodeA strains over time. FAME, fatty acid methyl esters. In panel D, the data represents the sum of all psi factor molecules detected in veA (= HODEs, PsiB1α and PsiC1α plus HOEs, PsiB1β and PsiC1β) and veA, ΔodeA (= HOEs). Determinations of fatty acid composition, including psi factor, were carried out at 42 h, 66 h, 114 h, 162 h and 240 h after inoculation on YGT medium. Cultures were grown at 26°C in the dark. Values are the means of three replicates.

Fig. 2. Deletion of odeA decreases colony growth (A) and conidial production (B). Cultures of veA1; veA1, ΔodeA; veA; and veA, ΔodeA strains were grown at 26°C in the dark, 37°C in the dark and 37°C in the light in glucose minimum medium. D, cultures grown in dark. L, cultures grown in light. Panel A, Five day old cultures. Panel B, 72 h old cultures. Values are the means of three replicates.

Fig. 3. Ascospore production. Panel A. Cultures of veA and veA, ΔodeA strains were grown at 26°C in the dark, 37°C in the dark and 37°C in the light for 240 h in YGT medium. D, cultures grown in dark. L, cultures grown in light. Panel B. Cultures of veA and veA, ΔodeA strains were grown at 26°C in the dark for 162 h in YGT medium. Values are the means of four replicates.

Fig. 4. Temperature-dependent and light-dependent expression of delta-12 desaturase (odeA) and the delta-9 desaturase (sdeA) genes from A. nidulans. Total RNA (20 µg) was isolated from mycelia after growing for 72 h on glucose minimum medium at different temperatures in a range from 40°C to 20°C. Light regulation was studied at 37°C. Panel A, veA strain. Panel B, veA, ΔodeA strain. Panel C, veA1 strain. Panel D, veA1, ΔodeA strain. D,
cultures grown in dark. L, cultures grown in light. An ethidium bromide stained picture of rRNA is shown to indicate RNA loading. Arrows indicate the accumulation of odeA and sdeA transcripts.

**Fig. 5.** Unsaturated fatty acid-mediated regulation of delta-12 desaturase (odeA) and delta-9 desaturase (sdeA) gene expression in *A. nidulans.* After growing the strains in glucose minimum medium (GMM) in liquid shaken cultures for 16 h, the mycelia were transferred to a different second medium: GMM, GMMLA (GMM plus sodium linoleate in tergitol), GMMOA (GMM plus sodium oleate in tergitol), GMMT (GMM plus tergitol), MM (minimum medium without glucose), MMLA (MM plus sodium linoleate in tergitol), MMOA (MM plus sodium oleate in tergitol) and MMT (MM with tergitol control). Total RNA (20 µg) was isolated from mycelia 8 h after the shift. Panel A, *veA.* Panel B, *veA*, ∆odeA strain. Panel C, *veAl* strain. Panel D, *veAl*, ∆odeA strain. An ethidium bromide stained picture of rRNA is shown to indicate RNA loading. The arrows indicate the accumulation of odeA and sdeA transcripts.
Table 1. Fungal strain used in this study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<td>FGCS26</td>
<td>(biA1; veA1)</td>
<td>FGSC*</td>
</tr>
<tr>
<td>FGSC89</td>
<td>(biA1; argB2; veA1)</td>
<td>FGSC</td>
</tr>
<tr>
<td>TAMC31.65</td>
<td>(biA1; veA1, ∆odeA)</td>
<td>this study</td>
</tr>
<tr>
<td>FGSC33</td>
<td>(biA1; pyroA4; veA1)</td>
<td>FGSC</td>
</tr>
<tr>
<td>RAMC25</td>
<td>(pyroA4; veA1, ∆odeA)</td>
<td>this study</td>
</tr>
<tr>
<td>TAMC33.1</td>
<td>(pyroA4; veA1, ∆odeA; odeA::pyroA)</td>
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<tr>
<td>RAMC28</td>
<td>(veA1)</td>
<td>this study</td>
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<tr>
<td>TAMC34</td>
<td>(pyroA4; veA1, ∆odeA; pyroA)</td>
<td>this study</td>
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<tr>
<td>WIM126</td>
<td>(pabaA1, yA2; veA)</td>
<td>L. Yager</td>
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<td>RAMC22.1</td>
<td>(biA1; veA)</td>
<td>this study</td>
</tr>
<tr>
<td>RAMC29.24</td>
<td>(biA1; veA, ∆odeA)</td>
<td>this study</td>
</tr>
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</table>

Table 2. Fatty acid composition of mycelia of ΔodeA and wild type in veA1 and veA genetic backgrounds.

<table>
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<tr>
<th>Sample</th>
<th>% FAME*</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>others</th>
</tr>
</thead>
<tbody>
<tr>
<td>veA1 26D</td>
<td>2.43±0.09</td>
<td>13.1±0.2</td>
<td>13.6±0.1</td>
<td>14.5±0.3</td>
<td>48.4±0.5</td>
<td>8.03±0.36</td>
<td>2.40±0.08</td>
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<td>veA1, ΔodeA26D</td>
<td>5.68±0.05</td>
<td>5.53±0.07</td>
<td>10.4±0.1</td>
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<td>veA 26D</td>
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<td>veA, ΔodeA26D</td>
<td>6.40±0.35</td>
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<td>0.62±0.08</td>
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<td>3.63±0.43</td>
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<tr>
<td>veA1 37D</td>
<td>1.81±0.16</td>
<td>15.4±0.06</td>
<td>8.90±0.19</td>
<td>15.0±0.3</td>
<td>55.7±0.2</td>
<td>1.77±0.13</td>
<td>3.18±0.44</td>
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<td>3.43±0.28</td>
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<td>veA1 37L</td>
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<td>3.04±0.16</td>
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<td>veA 37L</td>
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<td>5.16±0.20</td>
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<td>0.48±0.16</td>
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<td>3.24±0.08</td>
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</table>

The analysis was carried out on 72 h old mycelia grown in liquid glucose minimum medium under stationary conditions. FAME, fatty acid methyl esters. (*) weight percent FAME based on lyophilized weight of mycelia. Wt., weight. 16:0, palmitic acid. 18:0, stearic acid. 18:1, oleic acid. 18:2, linoleic acid. 18:3, linolenic acid. nd, not detected. tr, trace (below integration threshold). 26D, 26°C in the dark. 37D, 37°C in the dark. 37L, 37°C in the light. Values are means of three replicates. Standard deviation is shown.
Table 3. Psi composition of mycelia of ΔodeA and wild type in veA1 and veA genetic backgrounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hydroxy-FAME µg/g mycelium</th>
<th>8-HOE</th>
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<tr>
<td>veA1 26D</td>
<td>30.9±4.6</td>
<td>16.0±3.2</td>
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<tr>
<td>veA1, ΔodeA26D</td>
<td>299±24</td>
<td>nd</td>
<td>27.4±2.5*</td>
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<tr>
<td>veA 26D</td>
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<td>veA, ΔodeA37D</td>
<td>159±33</td>
<td>nd</td>
<td>tr</td>
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<td>veA1 37L</td>
<td>25.5±1.8</td>
<td>13.7±1.6</td>
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<tr>
<td>veA1, ΔodeA37L</td>
<td>252±96</td>
<td>nd</td>
<td>10.1±1.4*</td>
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<tr>
<td>veA 37L</td>
<td>25.2±6.1</td>
<td>15.0±0.8*</td>
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<td>veA, ΔodeA37L</td>
<td>121±33</td>
<td>nd</td>
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</table>

The analysis was carried out on 72 h old mycelia grown in liquid glucose minimum medium under stationary conditions. FAME, fatty acid methyl ester. 8-HOE, 8-hydroxy oleic acid = PsiB1β. 8-HODE, 8-hydroxy linoleic acid = PsiB1α. 5,8-diHOE, 5,8-dihydroxy oleic acid = PsiC1β. 5,8-diHODE, 5,8-dihydroxy linoleic acid = PsiC1α. 26D, 26°C in the dark. 37D, 37°C in...
the dark. 37L, 37°C in the light. nd, not detected. tr, trace (below detection threshold). Values are means of three replicates, except those labeled as (*), where n=2. Standard deviation is shown.
Genetic connection between fatty acid metabolism and sporulation in Aspergillus nidulans
Ana M. Calvo, Harold W. Gardner and Nancy P. Keller

J. Biol. Chem. published online May 14, 2001

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