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Biochemical and molecular evidence of a Δ9 fatty acid desaturase from *Ensifer meliloti* 1021

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ABSTRACT

It has been reported that *Ensifer meliloti* presents a high proportion of monounsaturated fatty acids and has a putative desaturase gene designated as *PhFAD12* (National Centre for Biotechnology Information), encoding a putative Δ12 desaturase-like protein. In this work, we report the desaturation capacity and characterisation of this gene encoding the putative fatty acid desaturase of *E. meliloti* 1021. This gene was also isolated from the rhizobial strain and overexpressed in *Escherichia coli*.

Compared to a control, the expression of this gene in the transformed strain decreased the levels of palmitic and stearic acids, enhanced palmitoleic and *cis*-vaccenic levels, and allowed for the detection of oleic acid. *E. coli* overexpressing the putative desaturase gene was capable of desaturating palmitic and stearic acids to monounsaturated fatty acids, similarly to the rhizobial strain. Our studies show that AAK64726 encodes a Δ9 desaturase instead of a Δ12 desaturase as previously indicated. This work describes evidence for the presence of a desaturase-mediated mechanism in monounsaturated fatty acid synthesis in *E. meliloti* 1021, which is modified by high growth temperature. This mechanism supplements the anaerobic mechanism for unsaturated fatty acid synthesis.

1. Introduction

Biological membranes are required to maintain the appropriate fluidity to support normal membrane structure and functions under various growth conditions. To achieve the correct physical state of the membrane lipids, bacteria stringently control the production of a variety of fatty acids (FA) with different melting temperatures (Mansilla et al., 2004). When adapting to an environment that requires more rigidity in membrane lipids, cells produce more saturated FA. When increased fluidity is needed, more unsaturated FA (UFA) are produced (Paulucci et al., 2011).

Understanding the mechanisms involved in the adaptive regulation of membrane lipid composition first requires the identification of the enzymes that mediate the critical steps in the synthesis of UFA before the factors that influence enzyme activities and expression levels can be uncovered (Macartney et al., 1994).

In bacteria, both anaerobic and aerobic mechanisms could be responsible for the synthesis of UFA. The anaerobic pathway, elucidated in detail for *Escherichia coli*, produces *cis*-vaccenic (18:1Δ11) FA by a specific 2,3-dehydratase acting at the C-10 level (Magnuson et al., 1993). This pathway is also present in rhizobia (Lopez-Lara

and Geiger, 2000). In addition, in certain bacteria, the introduction of double bonds into the FA may occur by a different mechanism, which is mediated by desaturases. This reaction is catalysed by oxygen-dependent desaturation of the full-length FA chain and requires a specific electron transport chain (Shanklin and Cahoon, 1998). The FA desaturases can be divided into two evolutionarily distinct classes: a class of soluble acyl-ACP desaturases and a class of membrane-bound enzymes, including acyl-CoA desaturase and acyl-lipid desaturase (Los and Murata, 1998). The membrane class is more widespread in nature than soluble class (López-Alonso et al., 2003). Published reports suggest that the integral membrane desaturases are utilised in bacteria more extensively than was initially thought. For example, the presence of a membrane-bound Δ9 desaturase in *Mycobacterium tuberculosis* has been experimentally verified (Phetsuksiri et al., 2003). Two desaturases have been described in *Pseudomonas aeruginosa*. These enzymes modify existing membrane phospholipid-associated FA and produce UFA from exogenous saturated FA, supplementing the anaerobic mechanism (Zhu et al., 2006). Recently, Li et al. (2009) reported the identification and functional characterisation of a membrane-bound Δ9 desaturase from the gram-negative marine bacterium *Pseudoalteromonas* sp. MLY15, which had highly functional Δ9 desaturase activity when expressed in *E. coli*. FA desaturases can also play an important role in maintaining UFA homeostasis in many organisms, which is achieved by feedback regulation (Aguilar and de Mendoza, 2006).

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Recently, we reported that in a peanut-nodulating rhizobia strain, the UFA synthesis pathway is supplemented by an aerobic mechanism mediated by a Δ9 desaturase, the expression of which can be downregulated by temperature to maintain membrane fluidity under stressful conditions (Paulucci et al., 2013). The sequence of this Δ9 desaturase protein has 99% identity with the sequence of a putative desaturase from *Ensifer meliloti* 1021 (GenBank accession # AAK64726). The putative desaturase gene of *E. meliloti* 1021 is designated as *PhFAD12* (National Centre for Biotechnology Information) and encodes a putative Δ12 desaturase-like protein. Although rhizobial nucleotide sequences, including those in *E. meliloti* 1021, have been submitted to public databases due to their sequence similarity to different putative FA desaturase genes, in most cases, the functions of these gene products have not yet been experimentally demonstrated. *E. meliloti* 1021 is a bacterium able to fix atmospheric nitrogen in symbiosis with the legume *Medicago sativa*. The ability of this bacterium to adapt to changing conditions in the soil is critical for survival. The identification and characterisation of desaturases in rhizobial microorganisms is very important because the presence of this type of enzymes may indicate an adaptive mechanism that could be used to improve the stability and retention of rhizobia within the inoculants.

To determine the function of the putative desaturase gene of *E. meliloti* 1021, this gene was heterologously expressed in *E. coli*, and the enzymatic activity of this FA desaturase was measured to demonstrate that the gene product was in its active form. This work shows that the function of the putative desaturase enzyme of *E. meliloti* 1021 is not that of a Δ12 desaturase, as had been indicated in the GenBank database, but instead functions as a Δ9 FA desaturase.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

Bacterial strains, plasmids and oligonucleotide primers used in this study are listed in Table 1.

E. meliloti 1021 was cultured at 28 °C or 37 °C in TY medium (Beringer, 1974) or in minimal medium (Robertsen et al., 1981). *E. coli* DH5α was used in all routine DNA manipulations and cloning procedures. *E. coli* BL21(DE3)pLysS was used as the host for the pET17b plasmid containing the putative desaturase gene from *E. meliloti* 1021. The recombinant *E. coli* strain was grown on Luria-Bertani (LB) broth containing the required antibiotics at 37 °C.

2.2. Incorporation of radioactive fatty acid

A total of 0.5 μCi of [1-^{14}C]palmitic (16:0), [1-^{14}C]stearic (18:0) or [1-^{14}C]oleic (18:1 Δ9) was added to 25 ml of culture. The cultures of rhizobial and transformed *E. coli* strains were incubated at appropriate temperatures with shaking for the appropriate time (24 h for *E. meliloti* 1021). The cells were then harvested by centrifugation at 6000 × *g* for 10 min at 4 °C. Pellets were washed twice with 0.9% NaCl and used for further studies.

3. Lipid extraction

Lipids were extracted from washed bacteria with chloroform/methanol/water (Bligh and Dyer, 1959). The lower phase, containing lipids, was dried under N$_2$ and dissolved in an appropriate volume of chloroform/methanol (2:1, by vol.).

3.1. Separation and quantification of radioactive fatty acids based on the degree of unsaturation

FA methyl esters (FAME) were prepared from total lipid extracts with 10% BF$_3$ in methanol (Morrison and Smith, 1964) and resolved according to the number of double bonds on TLC plates impregnated with AgNO$_3$ (10%, w/v), using hexane/ethyl ether/acetic acid (94:4:2, by vol.) as solvent. FAME bands were detected under UV light after spraying the plates with dichlorofluorescein, eluting (Henderson and Tocher, 1992) and drying in counting vials. Three millilitres Optiphase Hisafe 2 (Perkin–Elmer, USA) was added to each vial and radioactivity was measured by a liquid scintillation counter (Beckman LS 60001C, Brea, CA, USA) (Kates, 1972).

3.2. Cloning of the putative desaturase gene of E. meliloti 1021 and expression in E. coli

Two PCR primers, DES forward and DES reverse (Table 1), were designed to amplify the nucleotide sequence of the gene encoding the putative desaturase protein of *E. meliloti* 1021 (DES Em). Restriction sites (NotI and XhoI) were added at the 5′ end of each primer for cloning purposes (restriction sites are underlined). This gene was amplified by PCR, using genomic DNA of *E. meliloti* as a template. PCR amplification was carried out in a total volume of 25 μl containing 1 μl of template DNA, 0.2 μl of *Pfu* DNA polymerase ((3 U μl^{-1}) Promega, Madison, WI, USA), 2.5 μl of *Pfu* DNA polymerase 10× buffer, 0.5 μl of dNTPs (10 mM each) and 2.5 μl of primers (5 μM), under the following procedure: initial denaturation at 95 °C for 2 min, followed by 30 cycles of 95 °C for 1 min, 62 °C for 30 s, 72 °C for 4 min and a final extension at 72 °C for 5 min.

The resulting product was cloned into the pET17b expression vector. The expression construct for the putative gene, pET-*des Em*, (listed in Table 1) was used to transform *E. coli* BL21(DE3)pLysS by the method of Chung et al. (1989). Positive, ampicillin-resistant transformants screened on LB agar plates were further validated by PCR and subsequent sequencing.

The *E. coli* BL21(DE3)pLysS clone was subsequently used in overexpression experiments. Cells were grown overnight at 37 °C with shaking in LB medium (20 ml) supplemented with ampicillin (100 mg ml^{-1}). Culture aliquots were used to inoculate fresh media and were grown at 37 °C up to an OD$_{600}$ of 0.3–0.5. To induce gene expression, 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the cultures, which were grown for another 12 h prior to harvesting. Control experiments were performed under the same experimental conditions using *E. coli* transformants containing the empty plasmid pET17b. For FA composition experiments and experiments testing the activity of the overexpressed product on 18:0, cultures were supplemented with 400 μmol l^{-1} 18:0 at inoculation and collected after 12 h of induction.

3.3. Gel electrophoresis of recombinant protein of E. meliloti 1021.

The recombinant *E. coli* cells harbouring expression constructs were grown as explained above. Cells pelleted from a 50 ml culture were resuspended in 15 ml of buffer lysis that contained 20 mM Tris–HCl pH 8, 300 mM NaCl, 20 mM imidazole, 0.1% tween, 10% glycerol and 20 mM β-mercaptoethanol. The suspension was supplemented with 1 μg ml^{-1} DNase and 0.5% PMSF and cells were disrupted by ultrasonication. The homogenate was centrifuged at 29,000 × *g* for 30 min at 4 °C. The proteins contained in the supernatant were analysed by SDS-polyacrylamide gel electrophoresis (PAGE) on a 10% polyacrylamide gel. Protein bands were visualised by staining with Coomassie brilliant blue R-250.

Table 1
Strains, bacterial plasmid and oligonucleotide primers used in this study.

Strains, plasmid and primers	Description	Source or reference
Plasmids		
pET	pET17b vector expression	EMD biosciences
pET-*des Em*	pET17b harbouring putative desaturase gene of *E. meliloti 1021*	This study
Rhizobial strain		
Ensifer meliloti 1021	Rhizobial strain symbiont of *Medicago sativa*Sm^R of wild type SU47	Meade et al. (1982)
E. coli strain		
E. coli BL21(DE3)pLysS	F$^-$ ompT gal dcm lon hsdS$_B$(r$_B^-$ m$_B^-$) λ(DE3) pLysS(cm^R)	Davanloo et al. (1984)
E. coli pET	*E. coli* BL21(DE3)pLysS harbouring pET plasmid	This study
E. coli pET-des Em	*E. coli* BL21(DE3)pLysS harbouring pET-des Em plasmid	This study
Oligonucleotide primers		
DES forward	GCGGCCGCATGAGCGCACA NotI	Paulucci et al. (2013)
DES reverse	CTCGCGTTACTTCGTCTTTCGT XhoI	Paulucci et al. (2013)

3.4. Analysis of fatty acids by GC

FAME from *E. meliloti* 1021 and *E. coli* recombinant cells, prepared as above, were analysed using a Hewlett Packard 5890 II gas chromatograph (GC) equipped with a highly polar column (HP 88) of cyanopropyl (length 60 m; inner diameter 0.25 mm; film thickness 0.2 μm) and a flame ionisation detector. GC conditions were as follows: injector temperature of 250 °C, detector temperature of 300 °C, and nitrogen as the carrier gas. Temperature was programmed at 120 °C for 1 min and then increased by 10 °C min^{-1} to 175 °C for 10 min, 5 °C min^{-1} to 210 °C for 5 min and 5 °C min^{-1} to 230 °C for 5 min. The peak areas of carboxylic acids in total ion were used to determine relative amounts. Fatty acids were identified by comparison of retention times to commercial standards (Sigma Chemical Co., St. Louis, MO, USA).

3.5. Statistical analyses

Data were compared by one-way analysis of variance (ANOVA) test.

4. Results

4.1. Fatty acid composition of E. meliloti 1021

Major monounsaturated FA detected in *E. meliloti* 1021 were 18:1Δ11, 18:1Δ9 and small amounts of palmitoleic (16:1Δ9). The main saturated FA were 16:0, 18:0 and 19:0 cyclopropane (19:0$_{cycle}$) (Table 2).

4.2. Exogenous fatty acid desaturation on E. meliloti 1021

Fig. 1 shows the distribution of radioactivity among different FA fractions after incorporation of radioactive FA into cells of *E. meliloti* 1021. When [1-^{14}C]16:0 was added to cultures of *E. meliloti* 1021, a considerable amount of radioactivity was incorporated into

Table 2
Fatty acid composition of *E. meliloti 1021*strain.

	Ensifer meliloti 1021
Fatty acids (%)	
16:0	15.6 ± 0.9
16:1Δ9	1.30 ± 0.1
18:0	5.90 ± 0.3
18:1Δ11	62.7 ± 2.6
18:1Δ9	9.80 ± 0.9
19:0$_{cycle}$	4.70 ± 0.5

E. meliloti 1021 cells were cultivated 24 h at 28 °C in minimal medium. Total lipids were extracted, and total lipid FA were converted to methyl esters and analysed by GC as described in the text. The percentage of each fatty acid is relative to total FA (defined as 100%). Values represent means ± SEM of three independent experiments.

the monounsaturated FA fraction (52%). When [1-^{14}C]18:0 was added to the cell cultures, less radioactivity was recovered in the monounsaturated FA fraction (35.5%). The radioactivity stayed in the monounsaturated fraction when [1-^{14}C] 18:1 Δ9 was used as a substrate (data not shown).

4.3. Functional expression of the des Em gene in E. coli

To verify whether this activity is due to the product of a desaturase gene, we overexpressed the putative desaturase gene from *E. meliloti* 1021, *des Em*, in *E. coli*.

Fig. 2 shows the results of SDS-PAGE analysis of total proteins from *E. coli* cells that had been transformed with the pET-*des Em* plasmid and from cells that had been transformed with the empty pET plasmid. Induction with IPTG of the *des Em* gene resulted in the synthesis of a polypeptide with an apparent molecular mass of 50 kDa, as determined by SDS-PAGE.

To determine the function of the putative desaturase protein, radioactive FA were added to culture media. After addition of IPTG, the cultures of recombinant strains of *E. coli* were collected and their FA were analysed by TLC. Fig. 3 shows the distribution of radioactivity among different FA fractions after the incorporation of radioactive FA into *E. coli* pET and *E. coli* pET-des Em cells. When [1-^{14}C]16:0 was added to the cultures of strains transformed with the putative desaturase, a considerable amount of radioactivity was recovered from the monounsaturated FA fraction (20%), compared with the *E. coli* strain harbouring empty plasmid (1.3%) (Fig. 3A). When [1-^{14}C]18:0 was added to the cultures, we also found a significant amount of radioactivity in the monounsaturated FA fraction (12.6%), compared with the control strain (1.6%) (Fig. 3B).
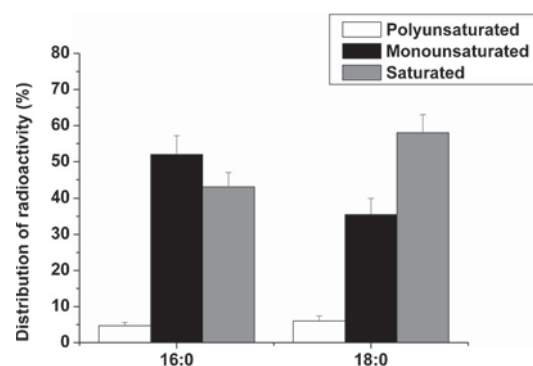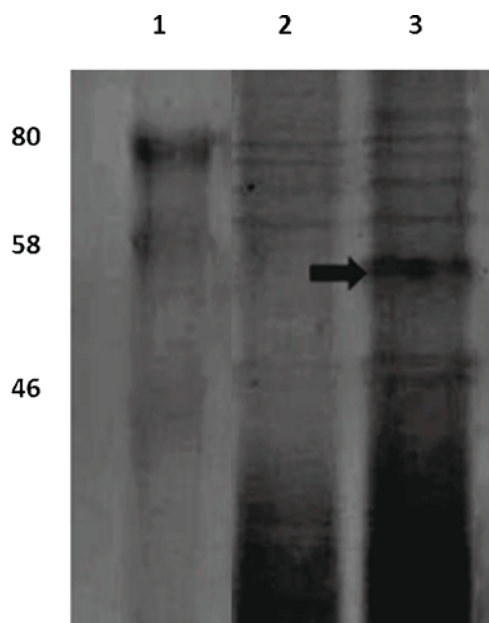

Fig. 1. *In vivo* desaturase activity measured in *E. meliloti* 1021. [1-^{14}C]16:0 or [1-^{14}C]18:0 FA were added to the cell culture in TY medium and incubated at 28 °C until late exponential phase. Total lipids were extracted, methanolysed and separated according to unsaturation degree using TLC plates impregnated with 10% AgNO$_3$. The results are expressed as a percentage of the total radioactivity incorporated in each FA fraction. The values represent the mean ± SEM from three independent experiments.
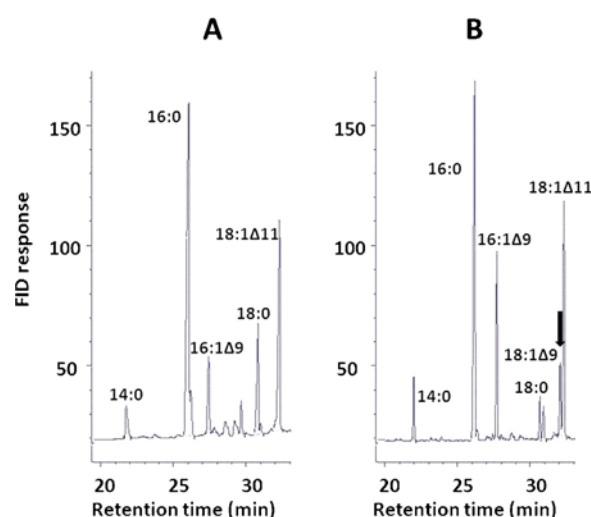
Fig. 2. SDS-PAGE analysis of recombinant protein expressed in *E. coli* after IPTG induction. IPTG at 0.1 mM was added to the growth medium for induction of *des Em*, and the culture was then incubated at 37 °C for 12 h before analysis of cellular proteins. Lane 1, prestained protein molecular mass marker; Lane 2, total protein of *E. coli* pET; Lane 3, total protein of *E. coli* pET-des Em. The arrow indicates recombinant protein.


Fig. 4. Gas chromatograms of methyl esters of fatty acids obtained from *E. coli* strains. The *E. coli* strains, containing the pET control (A) or pET-des Em (B) were cultivated at 37 °C for 12 h after induction with 0.1 mM IPTG. 18:0 was added to the media at a final concentration of 400 µM. The arrowhead indicates the position of oleic acid methyl ester.

Lower levels of radioactivity were recovered from the monounsaturated FA fraction when [1-^{14}C]18:0 was used as a substrate. In the transformed *E. coli* strain, the radioactivity stayed in the monounsaturated fraction when [1-^{14}C]18:1Δ9 was used as a substrate (data not shown).

Because radioactive substrates 16:0 and 18:0 were desaturated by the transformed *E. coli* strain that harbours the desaturase gene, we analysed its FA composition. After 12 h of IPTG-induction, the cultures of the transformed *E. coli* strain were collected and the FA composition was analysed by GC (Table 3). In *E. coli* pET, myristic acid (14:0), 16:0, 16:1Δ9, 18:0 and 18:1Δ11 were the main FA constituents found. In the *E. coli* pET-des Em strain, 16:0 levels decreased from 56.9% to 33.6%, while 16:1Δ9 levels increased from 9.4% to 23.0%. The changes in the pattern of 16:0 contents between the pET-des Em and the control strain indicated that the desaturase was capable of using 16:0 as a substrate. A 46% increase in the 18:1Δ11 content was observed in the pET-des Em strain. However, we were not able to detect 18:1Δ9. The lack of 18:1Δ9

production in the recombinant strain may have been a result of the lower level of the 18:0 substrate for the expressed enzyme. Therefore, the cultures were supplemented with 18:0 and collected, and their FA compositions were analysed by GC (Table 3). Under these conditions, the 18:0 content was reduced (66.7%), and we were able to detect 18:1Δ9 (5%) in cells transformed with the desaturase gene. Fig. 4 shows the results of GC analysis of FAME from the control (Fig. 4A) and the pET-des Em strain (Fig. 4B). An additional peak is visible in the trace obtained from the pET-des Em strain compared with the empty vector-transformed control. The retention time of this FA methyl derivative demonstrates that the novel peak is 18:1Δ9 methyl ester.

4.4. Effect of high growth temperature on fatty acid desaturation in E. meliloti 1021

Because the activity of the desaturase enzymes is often modified by the temperature in different organisms, we decided to measure exogenous FA desaturation at 37 °C in *E. meliloti*. When *E. meliloti* was grown at 37 °C (Fig. 5) and [1–^{14}C]16:0 was used as a substrate, monounsaturated FA fraction labelling decreased 57% relative to 28 °C. When [1–^{14}C]18:0 was used as a substrate, a 22.5% lower
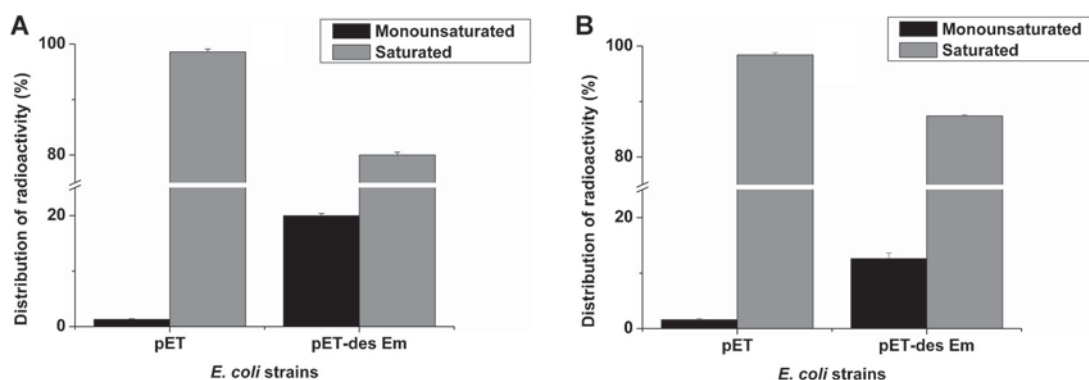
Fig. 3. *In vivo* desaturase activity measured in *E. coli* strain expressing putative desaturase gene. (A) [1-^{14}C]16:0 acid was added to the cell culture and incubated at 37 °C until an OD$_{600}$ of 0.3–0.5 was reached, after which the expression of recombinant proteins was induced by IPTG for 12 h. Total lipids were extracted, methanolysed and separated according to unsaturation degree using TLC plates impregnated with 10% AgNO$_3$. The results are expressed as a percentage of the total radioactivity incorporated in each FA fraction. (B) Same as panel A, except that the FA added to the culture was [1-^{14}C]18:0. Values represent mean $\pm$ SEM from three independent experiments.

Table 3

Fatty acid composition of *E. coli* strains.

E. coli strain	Fatty acids (%)											
	14:0		16:0		16:1Δ9		18:0		18:1Δ11		18:1Δ9	
	−18:0	+18:0	−18:0	+18:0	−18:0	+18:0	−18:0	+18:0	−18:0	+18:0	−18:0	+18:0
pET	6.6 ± 0.8	4.7 ± 0.5	56.9 ± 0.6	49.7 ± 1.0	9.4 ± 0.8	8.3 ± 0.9	1.4 ± 0.5	12.0 ± 0.9	25.7 ± 1.0	25.3 ± 1.2	ND	ND
pET-des Em	5.0 ± 0.7	3.5 ± 0.5	$33.6 \pm 1.7^{*}$	$44.5 \pm 1.6^{*}$	$23.0 \pm 1.5^{*}$	$17.0 \pm 2.0^{*}$	0.7 ± 0.3	$4.0 \pm 0.7^{*}$	$37.7 \pm 1.0^{*}$	26.0 ± 2.5	ND	$5.0 \pm 0.6^{*}$

Strains were grown at 37 °C on LB medium with (+) or without (−) 18:0 FA to an OD_{600} of 0.3–0.5, and the expression of recombinant protein was induced by the addition of IPTG. After an overnight induction (12 h), total lipids were extracted, and total lipid FA were converted to methyl esters and analysed by GC as described in the text. The percentage of each fatty acid is relative to total FA (defined as 100%). Values represent means ± SEM of three independent experiments.

[*] Difference from control (pET) value statistically significant at $P < 0.05$ level.

ND: not detected.

level of radioactivity was recovered from the monounsaturated FA fraction at 37 °C compared with 28 °C. Both substrates were desaturated lesser extent at 37 °C compared to 28 °C desaturation.

5. Discussion

The presence of Δ9 unsaturated FA in *E. meliloti* 1021, which was detected in this work and also in coincidence with Tighe et al. (2000), suggest the presence of a Δ9 desaturase enzyme. An aerobic mechanism in *E. meliloti* 1021 was demonstrated *in vivo* in this study using radioactive 16:0 or 18:0 as a substrate. This strain was able to desaturate radioactive substrates to a monounsaturated FA *in vivo*. Wada et al. (1989) demonstrated the existence of an aerobic pathway for the synthesis of monounsaturated FA in the *Pseudomonas* strain E-3 using radioactive FA as a substrate. In addition, they also reported that both mechanisms of FA synthesis are present in the *Pseudomonas* strain E-3. In another gram-negative bacterium, *Pseudoalteromonas*, a desaturase activity was also identified, but from more of a molecular biology approaches (Li et al., 2009). Nishida and Murata (1996) demonstrated that the expression of desaturase genes in heterologous hosts can help in understanding the mechanisms by which biological membranes adapt to temperature changes.

Wild type *E. coli* do not contain any FA desaturase (Cao et al., 2010) but do contain all of the complement systems needed for the measurement of desaturase enzyme activity. Therefore *E. coli* have been widely used for the expression of these enzymes. In this study, the *des Em* gene was successfully expressed in *E. coli* BL21(DE3)pLysS using the pET expression system, resulting in a 50 kDa protein. The desaturase activity of the recombinant protein could be readily detected when assayed *in vivo* with radioactive 16:0 or 18:0 FA as a substrate, both of which were converted to

Fig. 5. *In vivo* desaturase activity measured in *E. meliloti* 1021. [1-^{14}C]16:0 or [1-^{14}C]18:0 FA were added to the cell culture in TY medium and incubated at 37 °C until late exponential phase. Total lipids were extracted, methanolysed and separated according to unsaturation degree using TLC plates impregnated with 10% AgNO$_3$. The results are expressed as a percentage of the total radioactivity incorporated in each FA fraction. The values represent the mean ± SEM from three independent experiments.
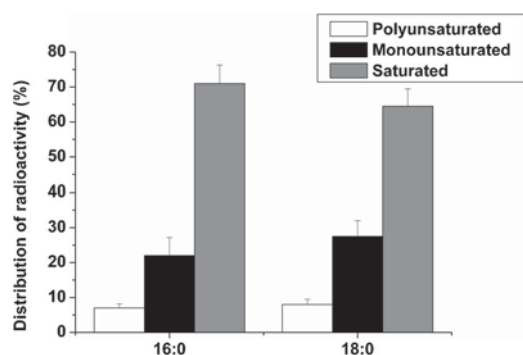
the corresponding monounsaturated FA. DES Em was able to use both FA as a substrate. This is also reflected in the FA composition of the recombinant strain, which displayed important decreases in 16:0 and 18:0, significant enhancements in the proportions of 16:1Δ9, 18:1Δ11 and the appearance of 18:1Δ9 FA, when 18:0 was added to the culture medium. These results are consistent with those of Cao et al. (2010) who found increased levels of 16:1Δ9 when a Δ9 desaturase of *Arabidopsis thaliana* was expressed in *E. coli*. They also found increased levels of 18:1Δ11, because the FA formed from 16:0, (16:1Δ9), can be elongated by FAS type II and converted into 18:1Δ11. We measured the *in vivo* desaturase activity in the strain of *E. coli* transformed with the *des Em* gene from the rhizobial strain *E. meliloti* 1021. Specifically, the transformed strain of *E. coli* was able to use both substrates (16:0 or 18:0), and the amount of radioactivity incorporated in the monounsaturated FA fraction was higher when 16:0 was added to the culture medium as a substrate than the incorporation with 18:0 as a substrate.

In this study, we also demonstrated that the desaturation of saturated FA was decreased at high growth temperature, causing a reduction in MUFA synthesis. These results are in coincidence with those obtained previously by us for *Bradyrhizobium* TAL1000 (Paulucci et al., 2013). Temperature-dependent changes in the ratio of unsaturated to saturated FA in membrane lipids are considered one of the regulatory mechanisms that maintain membrane fluidity (Shivaji and Prakash 2010). Increased incorporation of UFA decreases the melting temperature of membrane phospholipids, whereas increased incorporation of saturated FA has the opposite effect (Cronan and Rock 1996).

The genes and gene products involved in FA biosynthesis and metabolism need to be identified and characterised as a prerequisite for comprehensive understanding of these processes. In this biochemical and molecular study, we have shown that in *E. meliloti* 1021, another pathway in addition to FAS type II is present for the synthesis of monounsaturated FA. On the other hand, 18:1Δ9 was not used as a substrate by the native rhizobial strain nor by *E. coli* expressing the putative desaturase gene, suggesting that the putative desaturase of *E. meliloti* 1021 will not be a Δ12 desaturase. Instead, by detecting an increase of 16:1Δ9 and the appearance of 18:1Δ9 in the transformed *E. coli* strain, our results strongly suggest that the FA desaturase enzyme will be a Δ9 desaturase similar to *Bradyrhizobium* TAL100 (Paulucci et al., 2013). In *E. meliloti* 1021, the anaerobic UFA biosynthetic pathway is supplemented by an aerobic desaturase, and this alternative pathway provides a mechanism for modifying existing membrane phospholipid FA and producing UFA from exogenous saturated FA.

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