Supplementary data for article:

Ilic-Tomic, T.; Genčić, M. S.; Živković, M. Z.; Vasiljevic, B.; Djokic, L.; Nikodinovic-Runic, J.; Radulović, N. S. Structural Diversity and Possible Functional Roles of Free Fatty Acids of the Novel Soil Isolate Streptomyces Sp. NP10. *Applied Microbiology and Biotechnology* **2015**, *99* (11), 4815–4833. https://doi.org/10.1007/s00253-014-6364-5

Electronic Supplementary Material for:

Structural diversity and possible functional roles of free fatty acids of the novel soil isolate *Streptomyces* sp. NP10

Applied Microbiology and Biotechnology

Tatjana Ilic-Tomic[1,†], Marija S. Genčić[2,†], Milena Z. Živković[2], Branka Vasiljevic[1], Lidija Djokic[1], Jasmina Nikodinovic-Runic[1], Niko S. Radulović[2,*]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, P.O. Box 23, 11010 Belgrade, Serbia*

[2]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*

Table of Contents:

1. Supplementary Figures S1-S5

2. Supplementary Tables S1-S3

3. (EI) MS spectra of methyl esters of new FAs: *i*-17:0cy9-10 and *a*-18:0cy9-10

4. Identification methods of free FAs found in *Streptomyces* sp. NP10 strain

Fig. S1 Phenotypic characteristics of soil isolate *Streptomyces* sp. NP10: **A** cellulolytic activity using CMC cellulose in agar screen; **B** hemolytic activity on blood-agar and **C** ability to grow on in tryptone soy broth on a range of NaCl concentrations

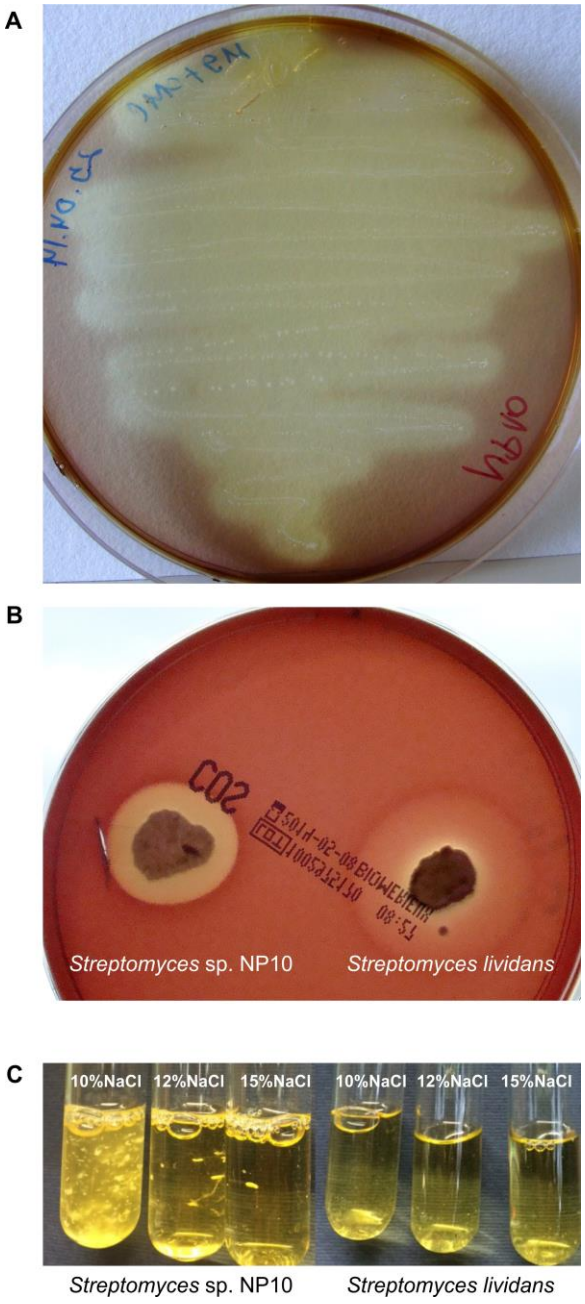
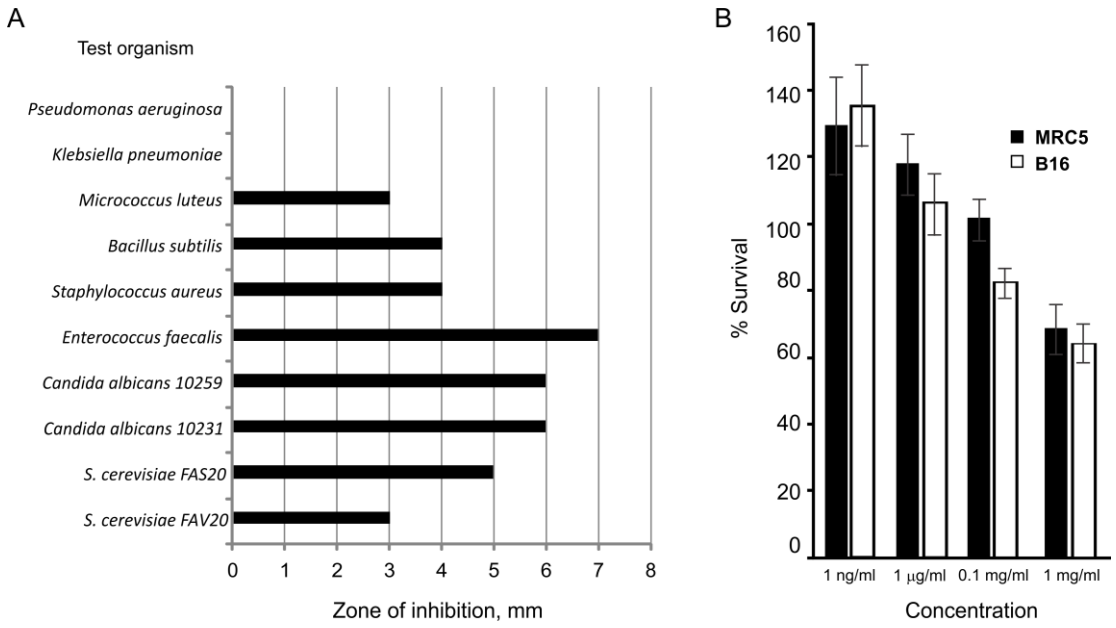


Fig. S2 Crude culture extracts of *Streptomyces* sp. NP10 showed **A** antimicrobial activity in disc-diffusion assay (200 µg per disc) and **B** exhibited mild cytotoxicity against human fibroblasts (MRC5) and melanoma (B16) cell lines judged from MTT assay. Kanamycin (25 µg per disc) caused inhibition zones 10-15 mm for bacterial strains, while nystatin (50 µg per disc) caused inhibition zones of 12 mm with *Candida* strains



Identification methods and diversity of free FAs found in *Streptomyces* sp. NP10 strain

The free FAs from the novel strain *Streptomyces* sp. NP10 were identified by GC-MS analysis as the corresponding methyl esters obtained after derivatization with CH_2N_2. All analyzed total ion chromatograms contained several series of FAMEs showing regularities in their GC retention behavior (constant retention index difference of *ca.* 100 units) and possessing analogous mass spectra. Normal chain FAMEs (Fig. 3a, marked red, main text) included all even and odd members of the homologue series from 7:0 to 26:0, as well as 28:0 and 30:0. These compounds were readily identified from their mass spectra by comparison to library spectra and subsequent GC-MS analysis of authentic standards. In general, mass spectra of normal chain FAMEs are characterized by the base peak at *m/z* 74 (Mc Lafferty rearrangement) and fragment ions at *m/z* 87 (β-cleavage) and $[M - 31]^+$ (loss of OCH_3 group), while all other fragment ions $[M - C_nH_{2n+1}]^+$ arose from cleavage of saturated unbranched alkyl chain (Dickschat et al. 2011; Fig. S3-a). Two more groups of saturated FAMEs showed mass spectra with significant fragment ions at *m/z* 74 and 87 but they eluted slightly faster from the GC column than the mentioned normal chain homologues implying that these were their branched-chain isomers (Fig. 3a, main text). Taking into account the so far established biosynthetic pathways by which bacteria could produce branched chain FAs it was assumed that these were methyl esters of methyl branched FAs (Kaneda 1991). The structures of these branched compounds were proposed based on a careful analysis of distinctions in their mass spectral fragmentation patterns since scission commonly occurs at adjacent bonds to the tertiary carbon atoms, yielding ions of variable intensities for different classes of isomers, as well as, on the decrease of retention indices (ΔRIs) compare to related normal chain isomer (Radulović et al. 2012). The first group of branched FAMEs had ΔRI of 36 units and relatively intensive fragment at $[M - 43]^+$ (loss of C_3H_7 group) which is indicative for *iso*-FAMEs (Radulović et al. 2012; Fig. S3-b). This assumption that (ω−1)-methyl branched series was in question was further supported by the occurrence of methyl esters of both even and odd FAs (from *i*-8:0 to *i*-20:0) (Fig. 3a, marked blue, main text) most probably derived from leucine or valine starters, respectively (Dickschat et al. 2011). Furthermore, as it was previously mentioned, the presence of the isopropyl moiety was clearly visible from the corresponding NMR spectra. Commercially available *i*:16:0 was then converted to methyl ester *via* CH_2N_2 and after GC co-injection, it was ambiguously confirmed that this synthetic compound was identical to the methyl ester of natural FAs. The second series of FAMEs had ΔRI value of 28 units and intense $[M - 57]^+$ ion (loss of C_4H_9) which is typical for *anteiso*-FAMEs (Radulović et al. 2012; Fig. S3-c). Within this group only the FAMEs originating from the odd FAs (starting from *a*-11:0 to *a*-19:0; Fig. 3a, marked magenta, main text) were found, pointing to an isoleucine-derived starter and additionally supporting methyl branching in the (ω−2)-position (Dickschat et al. 2011). Furthermore, the feature of (ω−1)-methyl branched compounds is that they elute slightly earlier (higher ΔRI value) than their (ω−2)-methyl branched counterparts. Recently we have found that for *iso*-alkane series ΔRI is 39 units (on DB-5MS column), while for *anteiso*-alkane is 30 units (Radulović et al. 2012), and this is in very nice agreement with presently established ΔRI values for *iso*-FAMEs (ΔRI = RI(*n*-ROOMe) – RI(*i*-ROOMe) = 36) and *anteiso*-FAMEs (ΔRI = RI(*n*-ROOMe) – RI(*a*-ROOMe) = 28). Then, the chemical shifts of carbon atoms from two initially non-identified methyl groups, at δ 11.430 and 19.234 ppm (Fig. 2c, main text), were almost identical with those reported for ω- and (ω – 2)-methyl groups (11.38 and 19.20 ppm, respectively) in synthetic 12-methyltetradecanoic acid (Biermann and Metzger 2004). Closer inspection of 1D and 2D NMR spectra and comparison of carbon chemical shifts with mentioned literature data enabled the detection of signals arrived from "CH_3-CH_2-$CH(CH_3)$-CH_2" structural fragment (Fig. 2b, main text).

These experimentally determined ΔRI values were further applied for estimation of branching methyl group position in methyl esters of some unsaturated and cyclopropane FAs. Precisely, a dozen of methyl esters of monounsaturated FAs, grouped again in three series according to their retention on GC column, were also detected. In contrast to the spectra of saturated FAMEs, hydrocarbon ions (with general formula $[C_nH_{2n-1}]^+$) dominate the spectrum of all detected monoenoic FAMEs, with ion at *m/z* 55 as the base peak, whereas the parent ion and fragment ions representing the loss of the methanol ($[M - 32]^{+\cdot}$), the loss of the Mc Lafferty ion ($[M - 74]^{+\cdot}$) and Mc Lafferty ion (at *m/z* 74) *per se* were also relatively abundant. While the identity of normal chain isomers, 16:1ω7c (at RI = 1899) and 18:1ω9c (RI = 2099), was undoubtedly confirmed by co-injection of authentic standards, for compounds eluting at RI 1863 and 2063 was assumed according to ΔRI value of 36 units (ΔRI = 1899 – 1863 and ΔRI = 2099 – 2063) that they were methyl esters of *iso*-monounsaturated FAs: *i*-16:1ω6c and *i*-18:1ω8c, respectively. Closer inspection of their mass spectra revealed also the presence of ions at $[M - 55]^+$, $[M - 87]^+$ and $[M - 105]^+$ indicative of the (ω-1)-position of the methyl group in branched monoenoic FAMEs (Boon et al. 1977).

Two more compounds eluting at RI 1971 and 2171 showed a similar set of fragment ions but shifted by 14 mass units to lower values at $[M - 69]^+$, $[M - 101]^+$ and $[M - 119]^+$ that together with ΔRI value of 28 units (*e.g.* ΔRI = 1999 – 1971) pointed to the (ω−2)-position of branching methyl group so they were identified as *a*-17:1ω7c and *a*-19:1ω9c, respectively (Boon et al. 1977). Since monounsaturated FAMEs, especially methyl-branched, are prone to double bond migration during GC-(EI)MS analysis (Rontani et al. 2009), they were further derivatized with DMDS in order to additionally confirm the double bond position. Generally the mass spectra of DMDS derivatives show strong fragmentation between the methylthio groups, and the fragment ion containing the methyl ester function is characterized by the further loss of CH_3OH (Dickschat et al. 2005). All EI-MS spectra of the herein analyzed DMDS adducts showed two substantial fragment ions at *m/z* 217 and $[M - 217]^+$. The third, relatively intense, peak was also observed at *m/z* 185 owing to the loss of CH_3OH from the ion at *m/z* 217 (Fig. 3b, main text). These data clearly indicated the double-bond position at C-9 in all detected monounsaturated FAs in the strain *Streptomyces* NP10. The presence of internal, non-conjugated, disubstituted double bond was also evident from NMR spectra of FR11 since two methines signals at δ 129.749 and 130.023 in ^{13}C NMR correlated to a ^1H NMR multiplet at δ 5.25-5.45 in the HSQC spectrum. Furthermore, these signals showed correlation in HMBC spectrum with appropriate ones for allylic methlylene proton and carbon atoms, at δ 2.021 ppm and 27.122 ppm, respectively (Fig. 2a, b and d, main text). The absolute value of the ^{13}C chemical shifts of allylic carbons could be by itself very diagnostic of the *cis* or *trans* configuration of a double bond since it was established that if double bond is close to mid-chain, the value of chemical shift of allylic carbons should be approximately 27 ppm for *cis* and 32 ppm for *trans* isomer (Santos and Graça 2014). Thus, the mentioned NMR data for FR11 are concordant with a *cis* configuration of double bond in all detected monoenoic FAs.

Additionally, one more group of FAMEs from fraction 11 was detected with mass spectral fragmentation identical to those described for monoenoic FAMEs, but having for a few units greater RI values, so it was assumed that these are methyl esters of cyclopropane FAs. In general, the identification of cyclopropane FAMEs by GC-MS analysis is hindered by the fact that their mass spectra are indistinguishable from those of monounsaturated FAs with one carbon longer alkyl chain since it appears that under electron bombardment cyclopropane ring rearrange to give a double bond (Christie and Holman 1966). However, RI values of methyl esters of monoenoic and cyclopropanoic FAs are sufficiently different and this can be useful information in deciding between possible structures. Our assumption that these were cyclopropane FAMEs was sustained by the fact that they remained the same upon derivatization with DMDS, while all monounsaturated FAMEs were converted to corresponding DMDS adducts. Thus, in order to clearly corroborate the presence of the cyclopropyl group, as well as, to assign its stereochemistry, the ^1H NMR spectrum, as well as other 1D and 2D NMR spectra, of non-derivatized FR11 were once again closely inspected. It was previously found that methylene protons of the cyclopropane ring in *cis* configuration normally show absorptions at *ca.* 0.6 ppm and at − 0.3 ppm, while in the case of a *trans* cyclopropane ring these two diastereotopic methylene hydrogens are similar, due to a pseudo-C_{2V} symmetry, and both resonate at approximately 0.2 ppm (Macmillan and Molinski 2005; Knothe 2006). Therefore, as protons of a cyclopropane ring normally resonate at high-field it could be possible to observe appropriate signals in the ^1H NMR spectrum of the whole FA mixture without interference from other peaks. The mentioned characteristic proton signals were indeed detected as *ddd* at δ 0.562 and quartet at δ − 0.333 ppm (while no absorption was observed at 0.2 ppm) and they showed HSQC cross peaks with the same secondary carbon atom (DEPT experiment) that resonated at 10.924 ppm in ^{13}C NMR spectrum. Additional broad singlet originating from the two methine protons was also observed at δ 0.644 ppm and it correlated with two methine carbons at 15.736 and 15.782 ppm (Fig. 2a, b and c, main text). These three high-field signals in the 1H NMR spectrum were also mutually coupled in homonuclear 2D spectra. All these observations are according to literature data indicative of the presence of a *cis* 1,2-disubstituted cyclopropane ring in the detected cyclopropane FAs (Macmillan and Molinski 2005; Knothe 2006). For compounds eluting at RI 2002 and 2202 it was assumed, based on comparisons of retention indices with literature values, that these are normal chain homologues 17:0cy9-10 and 19:0cy9-10, respectively (Zouari et al. 2011). Once again their identity was undoubtedly verified by co-injection of authentic samples obtained by cyclopropanation of methyl esters of 16:1ω7c and 18:1ω9c, respectively, using CH_2N_2 in the presence of $Pd(PhCN)_2Cl_2$ as the catalyst (Gangadhar et al. 1988), whereas the position of branching methyl group in *i*-17:0cy9-10 (RI = 1966) and *a*-18:0cy9-10 (RI = 2074) was inferred from the corresponding ΔRI values.

Finally, a group of minor compounds exhibiting mass spectra with two significant fragment ions at *m/z* 88 as the base peak and at *m/z* 101 pointed either to α-methyl branched FAMEs or fatty acid ethyl esters (FAEEs). The possibility of classifying these compounds in three series according to their RI values, as well as, the presence of [M

– 28]$^+$ ion (loss of CH_2=CH_2), arisen by Mc Lafferty rearrangement at the alkoxy branch of molecular ion, in their mass spectra (Gross 2004), prevailed on the side of ethyl esters of *n*, *iso*- and *anteiso*- FAs (*i*-14:0, *i*-15:0, *a*-15:0, *i*:16:0, 16:0 and 18:0). This assumption was confirmed by co-injection of authentic samples.

Several minor oxygenated FAs: 3-OH-8:0, 3-OH-10:0, 10-oxo-18:0, were identified as well. The identification was made easier by the fact that the introduction of hydroxy, oxo or epoxy functionality leads to very distinguishable fragmentation ions in mass spectra that also defines their position in the alkyl chain (*e.g*. 3-hydroxy-FAs were distinguished by the base peak at *m/z* 103 produced by a characteristic cleavage α to the carbon with the hydroxyl group, while intensive ion at *m/z* 155 was diagnostic for oxirane ring in position 9 and 10; Ryhage and Stenhagen 1960). Of course, wherever it was possible the initial identification was confirmed by co-injection of a synthetic standard.

Fig. S3 Mass spectra of **A** methyl pentadecanoate (15:0), **B** methyl 13-methyltetradecanoate (*i*-15:0) and **C** methyl 12-methyltetradecanoate (*a*-15:0) with distinguishing fragmentations

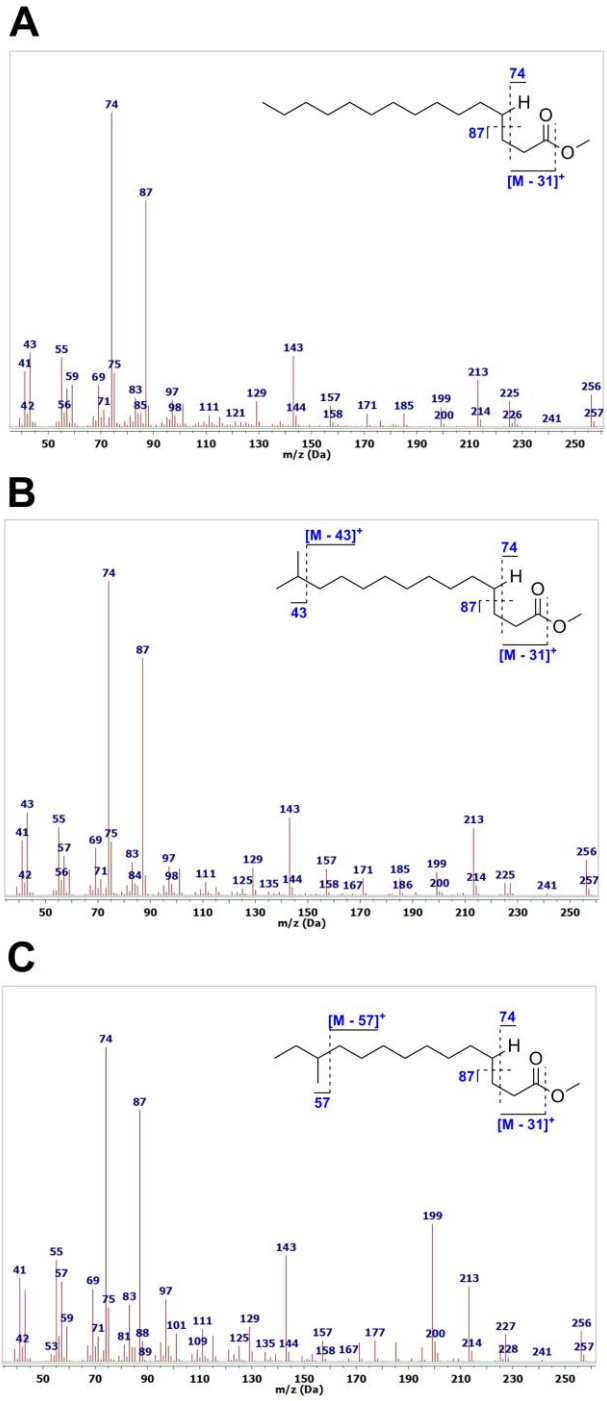


Fig. S4 Optimization of free fatty acids extraction from strain *Streptomyces* sp. NP10. **A** Distribution of major fatty acids. **B** Distribution of certain classes of fatty acids. Hex = hexane, EtOAc = ethyl acetate, Et_2O = diethyl ether and $CHCl_3$ = chloroform. N = normal chain, I = *iso*, A = *anteiso* and U = unsaturated FAs

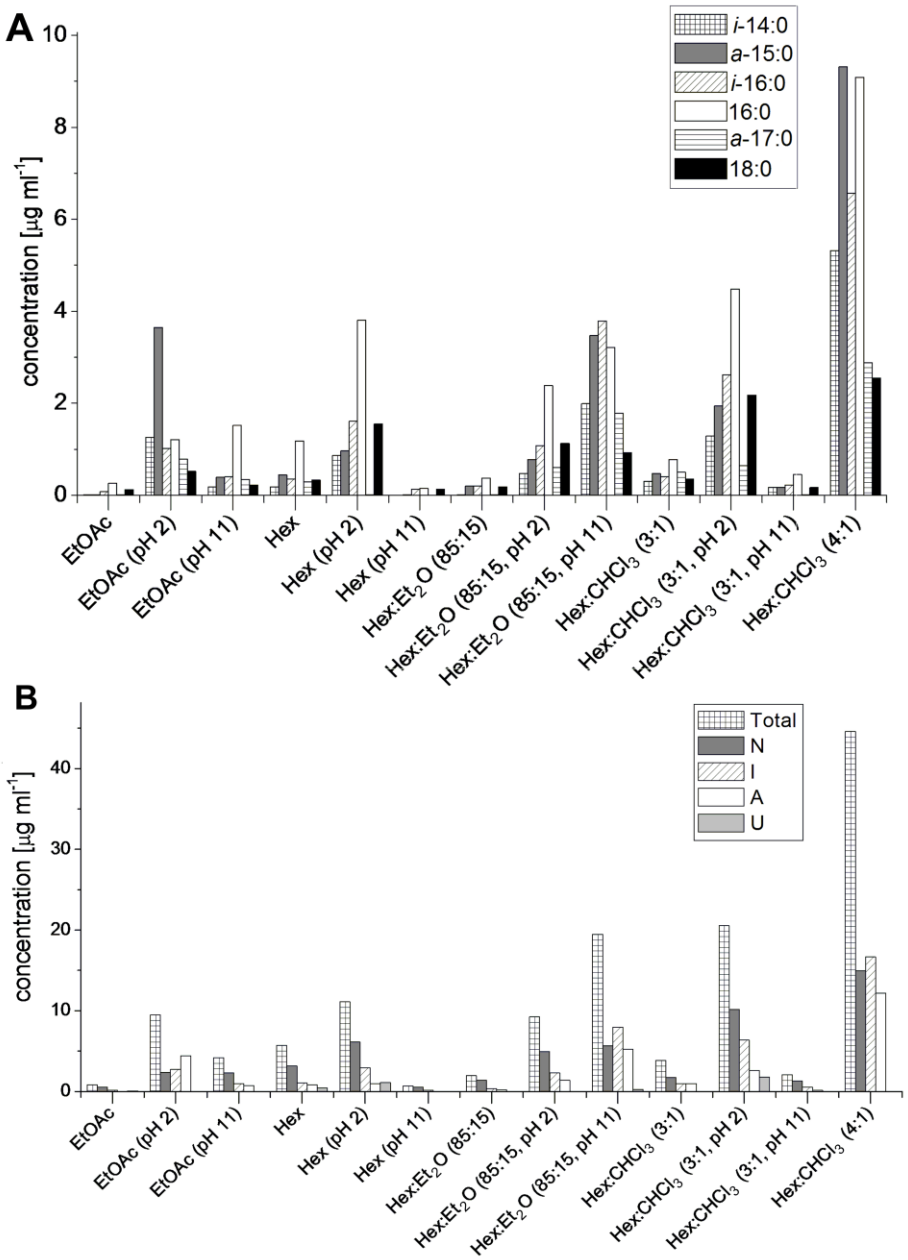
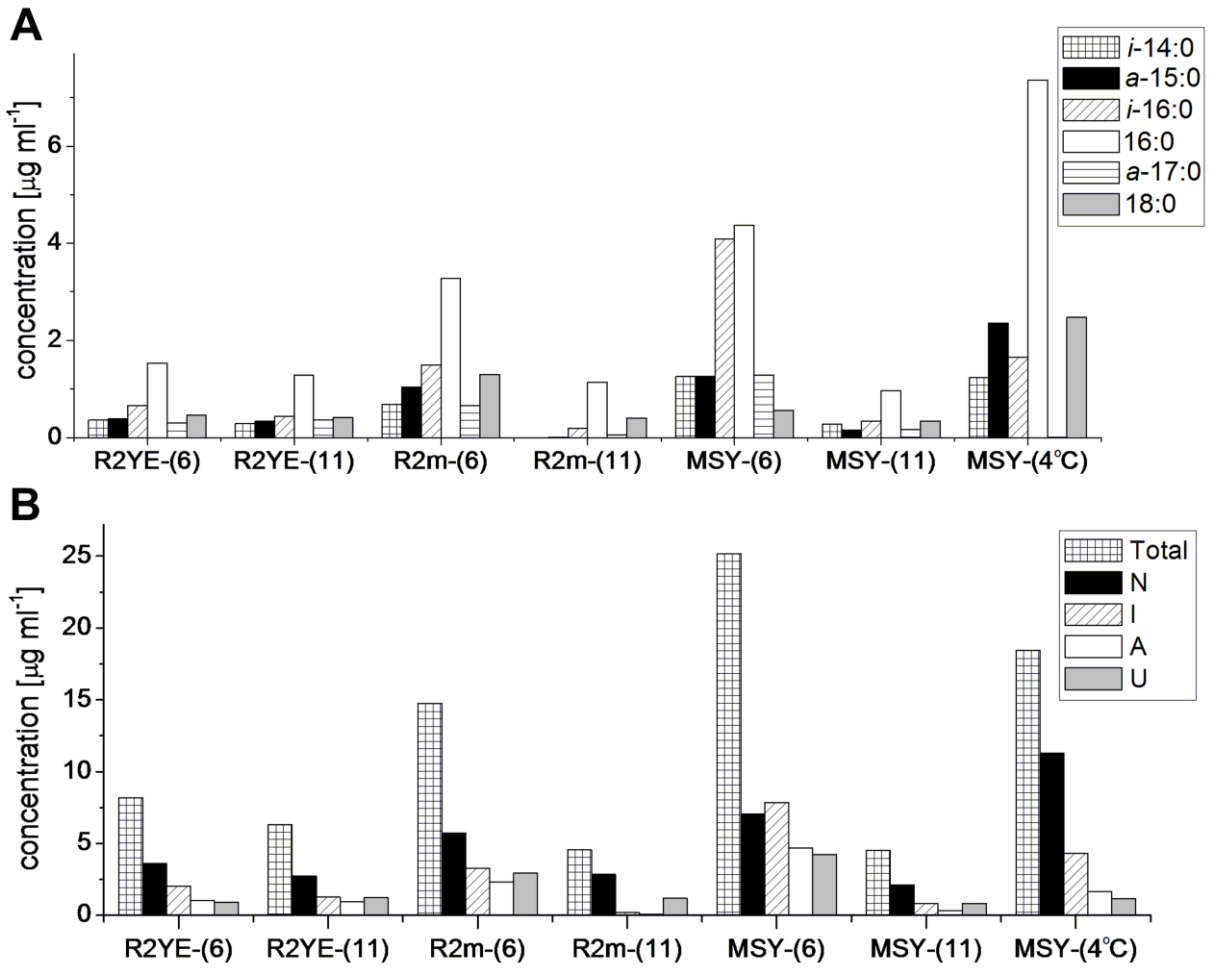


Fig. S5 Influence of different growth medium (R2YE* - minimal supplemented with yeast extract, R2m - defined minimal medium and MSY - maltose-soy flower) and temperature on production of free fatty acids by strain *Streptomyces* sp. NP10. **A**. Distribution of main fatty acids. **B.** Distribution of certain classes of fatty acids. Number in the brackets, except for last sample, represents the number of days after inoculation when the extraction was done. N = normal chain, I = *iso*, A = *anteiso* and U = unsaturated FAs



*R2m and R2YE (Kieser et al., 2000) contain per liter: 103 g sucrose, 0.25 g K_2SO4, 10.12 g $MgCl_2 \times 6H_2O$, 10 g glucose, 0.1 g Difco casamino acids, 0.05 g KH_2PO_4, 0.3 g L-proline, trace element solution 0.2 mL (per liter: 40 mg $ZnCl_2$, 200 mg $FeCl_3 \times 6H_2O$, 10 mg $CuCl_2 \times 2H_2O$, 10 mg $MnCl_2 \times 4H_2O$, 10 mg $Na_2B_4O_7 \times 10H_2O$ and 10 mg $(NH_4)_6Mo_7O_{24} \times 4H_2O$). In addition R2YE contains 0.5 g L^{-1} Difco yeast extract.

Table S1 Phenotypic characteristics of *Streptomyces* sp. NP10

Characteristics	**Result**
Growth on carbon or nitrogen source[a]	
glucose	+
mannitol	+
glycerol	+
sucrose	-
maltose	+
xylose	+
fructose	+
L-Alanine	+
L-Arginine	-
L-Asparagine	+
L-Cystine	-
L-Histidine	-
L-Lysine	+
Growth in the presence of	
NaCl (%, *w/v*)	12
thallous acetate[b]	+
sodium azide[b]	+
potassium telluride[b]	+
Biochemical activity	
starch hydrolysis	+
gelatin hydrolysis	+
urea hydrolysis	+
nitrate reduction	-
H_2S production	-
indole production	-
catalase presence	+
lipase presence	-
hemolysin presence	+
DNAse presence	+

[a] 0.1 %, *w/v*

[b] 0.001 %, *w/v*

Table S2 Cellular FA profile of new strain *Streptomyces* sp. NP10

RI[a]	Compound	Designation	Class	Content (%)		Method of identification[d]
				A[b]	B[c]	
1287	Methyl 8-methylnonanoate	*i*-10:0	I	tr[e]	tr	RI, MS
1323	Methyl decanoate	10:0	N	tr	tr	RI, MS, CoI
1388	Methyl 9-methyldecanoate	*i*-11:0	I	tr	0.4	RI, MS
1396	Methyl 8-methyldecanoate	*a*-11:0	A	tr	tr	RI, MS
1424	Methyl undecanoate	11:0	N	tr	tr	RI, MS, CoI
1488	Methyl 10-methylundecanoate	*i*-12:0	I	tr	0.7	RI, MS
1524	Methyl dodecanoate	12:0	N	tr	tr	RI, MS, CoI
1588	Methyl 11-methyldodecanoate	*i*-13:0	I	0.8	0.5	RI, MS
1596	Methyl 10-methyldodecanoate	*a*-13:0	A	1.3	0.8	RI, MS
1624	Methyl tridecanoate	13:0	N	0.5	0.4	RI, MS, CoI
1688	Methyl 12-methyltridecanoate	*i*-14:0	I	11.2	0.4	RI, MS
1699	Methyl (Z)-9-tetradecenoate	14:1ω5c	U	n.d.[f]	tr	RI, MS, DMDS
1724	Methyl tetradecanoate	14:0	N	2.7	2.3	RI, MS, CoI
1788	Methyl 13-methyltetradecanoate	*i*-15:0	I	6.2	0.8	RI, MS
1796	Methyl 12-methyltetradecanoate	*a*-15:0	A	16.3	0.4	RI, MS
1824	Methyl pentadecanoate	15:0	N	2.2	2.7	RI, MS, CoI
1863	Methyl (Z)-14-methylpentadec-9-enoate	*i*-16:1ω6c	U	tr	tr	RI, MS, DMDS
1888	Methyl 14-methylpentadecanoate	*i*-16:0	I	17.7	20.0	RI, MS, CoI
1899	Methyl (Z)-9-hexadecenoate	16:1ω7c	U	1.7	3.4	RI, MS, CoI, DMDS
1924	Methyl hexadecanoate	16:0	N	14.3	23.5	RI, MS, CoI
1971	Methyl (Z)-14-methylhexadec-9-enoate	*a*-17:1ω7c	U	3.4	2.5	RI, MS, DMDS

1988	Methyl 15-methylhexadecanoate	*i*-17:0	I	4.8	5.4	RI, MS
1996	Methyl 14-methylhexadecanoate	*a*-17:0	A	6.0	9.9	RI, MS
1999	Methyl (Z)-9-heptadecenoate	17:1ω8c	U	tr	tr	RI, MS, DMDS
2002	Methyl 8-(2-hexylcyclopropyl)octanoate	17:0cy9-10	CP	2.2	3.9	RI, MS, CoI
2024	Methyl heptadecanoate	17:0	N	1.2	2.0	RI, MS, CoI
2088	Methyl 16-methylheptadecanoate	*i*-18:0	I	2.5	1.0	RI, MS
2089	Methyl (Z,Z)-9,12-octadecadienoate	18:2ω6c	U	Tr	0.9	RI, MS, CoI
2099	Methyl (Z)-9-octadecenoate	18:1ω9c	U	2.0	7.0	RI, MS, CoI, DMDS
2124	Methyl octadecanoate	18:0	N	1.6	4.2	RI, MS, CoI
2188	Methyl 17-methyloctadecanoate	*i*-19:0	I	tr	tr	RI, MS
2196	Methyl 16-methyloctadecanoate	*a*-19:0	A	0.6	n.d.	RI, MS
2202	Methyl 8-(2-octylcyclopropyl)octanoate	19:0cy9-10	CP	0.3	0.7	RI, MS, CoI
2224	Methyl nonadecanoate	19:0	N	0.3	tr	RI, MS, CoI
2288	Methyl 18-methylnonadecanoate	*i*-20:0	I	0.3	n.d.	RI, MS
2291	Methyl *cis*-9,10-epoxystearate	18:0ep9-10c	EP	n.d.	4.7	RI, MS, CoI
2324	Methyl eicosanoate	20:0	N	tr	tr	RI, MS, CoI
2424	Methyl heneicosanoate	21:0	N	tr	n.d.	RI, MS
2432	Methyl 9,12-diepoxystearate (*isomer 1*)	18:0*di*-ep9-10:12-13	EP	n.d.	0.8	RI, MS, CoI
2462	Methyl 9,12-diepoxystearate (*isomer 2)*	18:0*di*-ep9-10:12-13	EP	n.d.	0.2	RI, MS, CoI
2488	Methyl 20-methylheneicosanoate	*i*-22:0	I	tr	tr	RI, MS
2524	Methyl docosanoate	22:0	N	tr	0.1	RI, MS, CoI
2578	Methyl 9,10-dihydroxynonadecenoate(*isomer 1*)	9,10-*di*-OH-19:1	H	n.d.	0.2	MS
2586	Methyl 9,10-dihydroxynonadecenoate(*isomer 2*)	9,10-*di*-OH-19:1	H	n.d.	0.2	MS
2724	Methyl tetracosanoate	24:0	N	n.d.	tr	RI, MS
	Total			100 (40)	100 (42)	
	Saturated fatty acid methyl esters			90.4 (32)[g]	75.5 (28)	

Normal chain (N)	22.6 (13)	35.3 (13)
even-numbered	18.5 (7)	30.1 (8)
odd-numbered	4.1 (6)	5.2 (5)
Iso (I)	43.5 (12)	29.1 (10)
even-numbered	31.7 (7)	22.1 (5)
odd-numbered	11.8 (5)	7.0 (5)
Anteiso (A)	24.3 (5)	11.1 (4)
even-numbered	n.d.	n.d.
odd-numbered	24.3 (5)	11.1 (4)
Unsaturated fatty acid methyl esters (U)	7.1 (6)	13.8 (7)
normal chain	3.7 (4)	11.3 (5)
iso	tr (1)	tr (1)
anteiso	3.4 (1)	2.5 (1)
Hydroxy fatty acid methyl esters (H)	n.d.	0.4 (2)
Epoxy fatty acid methyl esters (EP)	n.d.	5.7 (3)
Cyclopropane fatty acid methyl esters (CP)	2.5 (2)	4.6 (2)

[a] RI − Retention indices on a DB-5 column calculated against a series of co-injected *n*-alkanes (C_6–C_{34})

[b] Ethyl acetate whole cell extract of strain *Streptomyces* sp. NP10

[c] Ethyl acetate (pH 2) whole cell extract of strain *Streptomyces* sp. NP10

[d] RI − Constituent identified by retention index matching; MS − Constituent identified by mass spectra comparison; CoI − The identity of the constituent was additionally confirmed by co-injection of an authentic sample; DMDS – Position of double bond was confirmed by formation of corresponding dimethyldisulfide adducts

[e] tr − trace (<0.05%)

[f] n.d. – not detected

[g] number in brackets represents the number of compounds belonging to that class

Table S3. Free FAs profiles of four different *Streptomyces* strains

RI[a]	Compound	Designation	Class	*Streptomyces* sp. NP10	*Streptomyces* sp. NP2	*Streptomyces durmitorensis*	*Streptomyces lividans*	Method of identification[b]
1022	Methyl heptanoate	7:0	N	tr[c]	n.d.	n.d.	n.d.[d]	RI, MS, CoI
1122	Methyl octanoate	8:0	N	0.039[e]	n.d.	n.d.	n.d.	RI, MS, CoI
1222	Methyl nonanoate	9:0	N	0.090	tr	n.d.	tr	RI, MS, CoI
1255	Methyl 3-hydroxyoctanoate	3-OH-8:0	H	tr	n.d.	n.d.	n.d.	RI, MS
1287	Methyl 8-methylnonanoate	*i*-10:0	I	tr	n.d.	n.d.	n.d.	RI, MS
1323	Methyl decanoate	10:0	N	tr	tr	tr	tr	RI, MS, CoI
1388	Methyl 9-methyldecanoate	*i*-11:0	I	n.d.	n.d.	n.d.	tr	RI, MS
1396	Methyl 8-methyldecanoate	*a*-11:0	A	tr	n.d.	n.d.	tr	RI, MS
1404	Methyl 10-undecenoate	11:1ω1	U	tr	n.d.	n.d.	tr	RI, MS, CoI
1424	Methyl undecanoate	11:0	N	tr	tr	n.d.	n.d.	RI, MS, CoI
1488	Methyl 10-methylundecanoate	*i*-12:0	I	n.d.	tr	n.d.	0.023	RI, MS
1524	Methyl dodecanoate	12:0	N	n.d.	0.026	0.037	0.057	RI, MS, CoI
1588	Methyl 11-methyldodecanoate	*i*-13:0	I	0.066	0.018	n.d.	tr	RI, MS
1596	Methyl 10-methyldodecanoate	*a*-13:0	A	tr	0.017	n.d.	tr	RI, MS
1624	Methyl tridecanoate	13:0	N	0.036	n.d.	n.d.	tr	RI, MS, CoI
1688	Methyl 12-methyltridecanoate	*i*-14:0	I	1.174	0.263	0.093	0.170	RI, MS
1724	Methyl tetradecanoate	14:0	N	0.287	0.046	0.054	0.135	RI, MS, CoI
1753	Ethyl 12-methyltridecanoate		E	tr	tr	tr	tr	RI, MS
1788	Methyl 13-methyltetradecanoate	*i*-15:0	I	0.586	0.153	0.205	0.437	RI, MS
1796	Methyl 12-methyltetradecanoate	*a*-15:0	A	2.057	0.481	0.420	0.431	RI, MS
1824	Methyl pentadecanoate	15:0	N	0.164	0.031	0.035	0.128	RI, MS, CoI
1853	Ethyl 13-methyltetradecanoate		E	tr	tr	tr	tr	RI, MS
1861	Ethyl 12-methyltetradecanoate		E	tr	tr	tr	tr	RI, MS, CoI
1863	Methyl (*Z*)-14-methylpentadec-9-enoate	*i*-16:1ω6c	U	tr	tr	0.031	0.172	RI, MS, DMDS
1888	Methyl 14-methylpentadecanoate	*i*-16:0	I	1.449	0.576	0.544	0.893	RI, MS, CoI

1899	Methyl (*Z*)-9-hexadecenoate	16:1ω7c	U	0.175	0.169	0.046	0.329	RI, MS, CoI, DMDS
1924	Methyl hexadecanoate	16:0	N	2.006	0.551	0.130	0.619	RI, MS, CoI
1953	Ethyl 14-methylpentadecanoate		E	tr	tr	tr	n.d.	RI, MS, CoI
1966	Methyl 8-(2-(4-methylpentyl)cyclopropyl)octanoate	*i*-17:0cy9-10	CP	tr	0.044	0.053	n.d.	RI, MS
1971	Methyl (*Z*)-14-methylhexadec-9-enoate	*a*-17:1ω7c	U	tr	tr	0.064	0.424	RI, MS, DMDS
1988	Methyl 15-methylhexadecanoate	*i*-17:0	I	0.314	0.061	0.106	0.205	RI, MS
1989	Ethyl hexadecanoate		E	n.d.	tr	tr	tr	RI, MS, CoI
1996	Methyl 14-methylhexadecanoate	*a*-17:0	A	0.637	0.123	0.293	0.319	RI, MS
1999	Methyl (*Z*)-9-heptadecenoate	17:1ω8c	U	tr	n.d.	n.d.	tr	RI, MS, DMDS
2002	Methyl 8-(2-hexylcyclopropyl)octanoate	17:0cy9-10	CP	0.177	0.094	0.136	0.228	RI, MS, CoI
2024	Methyl heptadecanoate	17:0	N	0.114	0.027	0.035	0.041	RI, MS, CoI
2053	Ethyl 15-methylhexadecanoate		E	n.d.	tr	tr	tr	RI, MS
2061	Ethyl 14-methylhexadecanoate		E	tr	tr	tr	tr	RI, MS
2063	Methyl (*Z*)-16-methylheptadec-9-enoate	*i*-18:1ω8c	U	tr	tr	tr	tr	RI, MS, DMDS
2074	Methyl 8-(2-(4-methylhexyl)cyclopropyl)octanoate	*a*-18:0cy9-10	CP	tr	0.041	0.041	tr	RI, MS
2088	Methyl 16-methylheptadecanoate	*i*-18:0	I	0.089	tr	tr	tr	RI, MS
2089	Methyl (*Z*,*Z*)-9,12-octadecadienoate	18:2ω6c	U	n.d.	n.d.	n.d.	tr	RI, MS, CoI
2099	Methyl (*Z*)-9-octadecenoate	18:1ω9c	U	tr	0.194	0.044	0.118	RI, MS, CoI, DMDS
2124	Metyl octadecanoate	18:0	N	0.563	0.080	0.062	tr	RI, MS, CoI
2195	Ethyl octadecanoate		E	tr	tr	tr	tr	RI, MS, CoI
2202	Methyl 8-(2-octylcyclopropyl)octanoate	19:0cy9-10	CP	tr	0.018	n.d.	tr	RI, MS
2224	Methyl nonadecanoate	19:0	N	tr	tr	n.d.	tr	RI, MS, CoI
2324	Methyl eicosanoate	20:0	N	tr	0.016	tr	tr	RI, MS, CoI
2424	Methyl heneicosanoate	21:0	N	n.d.	tr	tr	tr	RI, MS
2524	Methyl docosanoate	22:0	N	tr	0.016	tr	tr	RI, MS, CoI
2624	Methyl tricosanoate	23:0	N	tr	tr	tr	tr	RI, MS
2724	Methyl tetracosanoate	24:0	N	n.d.	tr	n.d.	tr	RI, MS
2924	Methyl hexacosanoate	26:0	N	tr	tr	n.d.	tr	RI, MS
3024	Methyl heptacosanoate	27:0	N	tr	n.d.	n.d.	n.d.	RI, MS

3124	Methyl octacosanoate	28:0	N	tr	n.d.	n.d.	tr	RI, MS
	Total			10.021 (47)	3.044 (43)	2.427 (34)	4.731 (47)	
	Saturated fatty acid methyl esters			9.671 (29)[f]	2.485 (26)	2.014 (18)	3.458 (29)	
	Normal chain (N)			3.299 (18)	0.793 (16)	0.353 (11)	0.980 (17)	
	even-numbered			2.895 (9)	0.735 (9)	0.283 (7)	0.811 (10)	
	odd-numbered			0.404 (9)	0.058 (7)	0.070 (4)	0.169 (7)	
	Iso (I)			3.678 (7)	1.071 (7)	0.948 (5)	1.728 (8)	
	even-numbered			2.712 (4)	0.839 (4)	0.637 (3)	1.086 (4)	
	odd-numbered			0.966 (3)	0.232 (3)	0.311 (2)	0.642 (4)	
	Anteiso (A)			2.694 (4)	0.621 (3)	0.713 (2)	0.750 (4)	
	even-numbered			n.d.	n.d.	n.d.	n.d.	
	odd-numbered			2.694 (4)	0.621 (3)	0.713 (2)	0.750 (4)	
	Unsaturated fatty acid methyl esters (U)			0.175 (7)	0.407 (5)	0.174 (5)	0.619 (7)	
	normal chain			0.175 (4)	0.363 (2)	0.090 (2)	0.447 (5)	
	iso			tr (2)	tr (2)	0.031 (2)	0.172 (2)	
	anteiso			tr (1)	0.044 (1)	0.053 (1)	n.d.	
	3-Hydroxy fatty acid methyl esters (H)			tr (1)	n.d.	n.d.	n.d.	
	Cyclopropane fatty acid methyl esters (CP)			0.177 (4)	0.153 (4)	0.241 (3)	0.652 (4)	
	Saturated fatty acid ethyl esters (E)			tr (6)	tr (8)	tr (8)	tr (7)	

[a] RI − Retention indices on a DB-5 column calculated against a series of co-injected *n*-alkanes (C_6–C_{34})

[b] RI − Constituent identified by retention index matching; MS − Constituent identified by mass spectra comparison; CoI − The identity of the constituent was additionally confirmed by co-injection of an authentic sample; DMDS – Position of double bond was confirmed from the fragmentation pattern of the corresponding dimethyl disulphide adducts

[c] tr − trace (<0.015 μg mg^{-1})

[d] n.d. – not detected

[e] concentration is expressed as μg per mg of the dry mycelium

[f] number in brackets represents the number of compounds belonging to that class

Methyl 8-(2-(4-methylpentyl)cyclopropyl)octanoate: RI (DB-5) = 1966; MS(EI, 70 eV), m/z (rel. int, %): 282 (2.7) $[M]^{+\cdot}$, 250 (22.1) ($[M - CH_3OH]^+$, 208 (9.4), 227 (10.5), 195 (7.8), 177 (7.3), 166 (7.1), 152 (8.9), 139 (12),137 (11.2), 123 (18.4), 111 (27), 97 (51.4), 87 (41.6), 83 (60.5), 74 (56.9), 70 (53.2), 69 (92), 57 (39.3), 55 (100), 43 (50.7), 41 (61.4).

Methyl 8-(2-(4-methylhexyl)cyclopropyl)octanoate: RI (DB-5) = 1966; MS(EI, 70 eV), m/z (rel. int, %): 296 (0.8) $[M]^{+\cdot}$, 264 (16.7) ($[M - CH_3OH]^+$, 227 (17.8), 222 (4.8), 195 (11.7), 177 (9.8), 165 (4.6), 153 (9.7), 139 (11.4),137 (9.5), 123 (15.2), 111 (24.9), 97 (51.9), 87 (30.5), 83 (65.4), 74 (43), 70 (100), 69 (69.6), 57 (39), 55 (95), 43 (37.7), 41 (54.1).

References

Biermann U, Metzger JO (2004) Alkylation of alkenes: ethylaluminum sesquichloride-mediated hydro-alkyl additions with alkyl chloroformates and di-*tert*-butylpyrocarbonate. J Am Chem Soc 126:10319–10330. doi: 10.1021/ja048904y

Boon JJ, van de Graaf B, Schuyl PJW, de Lange F, de Leeuw JW (1977) The mass spectrometry of *iso* and *anteiso* monoenoic fatty acids. Lipids 12:717–721. doi: 10.1007/BF02570901

Christie WW, Holman RT (1966) Mass spectrometry of lipids. I. Cyclopropane fatty acid esters. Lipids 1:176–182. doi: 10.1007/BF02531869

Dickschat JS, Bode HB, Kroppenstedt RM, Müller R, Schulz S (2005) Biosynthesis of *iso*-fatty acids in myxobacteria. Org Biomol Chem 3:2824–2831. doi: 10.1039/B504889C

Dickschat JS, Bruns H, Riclea R (2011) Novel fatty acid methyl esters from the actinomycete *Micromonospora aurantiaca*. Beilstein J Org Chem 7:1697–1712. doi: 10.3762/bjoc.7.200

Gangadhar A, Subbarao R, Lakshminarayana G (1988) Cyclopropanation of unsaturated fatty acid methyl esters using diazomethane and palladium(II) acetate. J Am Oil Chem Soc 65:601–606. doi: 10.1007/BF02540687

Gross JH (2004) Mass spectrometry. A textbook. Springer, Berlin-Heidelberg

Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical Streptomyces Genetics. The John Innes Foundation, Norwich

Knothe G (2006) NMR characterization of dihydrosterculic acid and its methyl ester. Lipids 41:393–396. doi: 10.1007/s11745-006-5110-x

Macmillan JB, Molinski TF (2005) Majusculoic acid, a brominated cyclopropyl fatty acid from a marine cyanobacterial mat assemblage. J Nat Prod 68:604–606. doi: 10.1021/np049596k

Radulović N, Denić M, Stojanović-Radić Z, Skropeta D (2012) Fatty and volatile oils of the gypsywort *Lycopus europaeus* L. and the Gaussian-like distribution of its wax alkanes. J Am Oil Chem Soc 89:2165–2185. doi: 10.1007/s11746-012-2118-7

Rontani J-F, Zabeti N, Aubert C (2009) Double bond migration to methylidene positions during electron ionization mass spectrometry of branched monounsaturated fatty acid derivatives. J Am Soc Mass Spectr 20:1997–2005. doi: 10.1016/j.jasms.2009.07.020

Ryhage R, Stenhagen E (1960) Mass spectrometric studies. VI. Methyl esters of normal chain oxo-, hydroxy-, methoxy- and epoxy-acids. Ark Kemi 15:545–574.

Santos S, Graça J (2014) Stereochemistry of C_{18} monounsaturated cork suberin acids determined by spectroscopic techniques including ^{1}H-NMR multiplet analysis of olefinic protons. Phytochem Anal 25:192–200. doi: 10.1002/pca.2491

Zouari N, Fakhfakh N, Zouari S, Sellami M, Abid M, Ayadi MA, Zaidi S, Neffati M (2011) Volatile and lipid analyses by gas chromatography/mass spectrometry and nutraceutical potential of edible wild *Malva aegyptiaca* L. (Malvaceae). Int J Food Sci Nutr 62:600–608. doi: 10.3109/09637486.2011.564157