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

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Abstract Herein, a novel soil bacterium *Streptomyces* sp. NP10 able to grow outside usual streptomycetes optimum conditions (e.g., at 4 °C, pH 9 and high NaCl concentration), exhibiting atypical hemolytic, DNAse, and cellulolytic activities, is described. This strain produces and excretes into the growth medium large amounts of free long-chain fatty acids (FAs). A concurrent lipidomics study revealed a large structural diversity of FAs with over 50 different *n*- and branched-chain, (un)saturated, and cyclopropane FAs (C_7–C_{30}) produced by this strain. Two of these, *i*-17:0cy9-10 and *a*-18:0cy9-10, represent new natural products and the first ever identified branched cyclopropane FAs. Both free and bound lipid profiles of *Streptomyces* sp. NP10 were dominated by saturated branched chain FAs (*i*-14:0, *a*-15:0, and *i*-16:0). Although these free FAs showed only a moderate antimicrobial activity, our results suggest that they could have an ecophysiological role in interspecies signaling with another soil microorganism *Pseudomonas aeruginosa*. This work represents the first comprehensive report on the structural diversity and complexity of the free FA pool in *Streptomyces*. A naturally occurring streptomycete, such as *Streptomyces* sp. NP10, which secretes significant amounts of free long-chain FAs (non-cytotoxic) into the medium, could be useful in microbial biodiesel production.

Keywords *Streptomyces* sp. NP10 · Lipidomics · Free fatty acids · Branched cyclopropane fatty acids · Ecophysiological role · Microbial biodiesel production

Introduction

Members of the genus *Streptomyces* are renowned for their morphological complexity as well as their capacity to produce an unrivaled range of important metabolites, including half of all known clinically used antibiotics, as well as a number of anticancer, immunosuppressive, anthelmintic, and antifungal agents (Hopwood 1988; van Wezel and McDowall 2011). *Streptomyces* spp. commonly inhabit soil, one of the most microbially diverse environments on earth (Hibbing et al. 2010). In order to survive in such a complex ecosystem, streptomycetes, as non-motile organisms, had to adopt and find a way to face with innumerable stresses (chemical, physical, and/or biological) that could occur. It is established that streptomycetes are particularly vulnerable to competition from other soil microorganisms during the transition phase in colonial development when the growth of the vegetative mycelium is slowing as a result of nutrient exhaustion and the aerial mycelium is about to develop at the expense of nutrients released by breakdown of the vegetative hyphae (Hopwood 1988; Challis and Hopwood 2003). Under this selective pressure, streptomycetes typically stimulate the production of chemically diverse metabolites. Thus, it is believed that the ecological function of many *Streptomyces* metabolites is in the defense of the food source when other soil microorganisms threaten it, when they act as antibiotics that thwart the growth

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of microorganisms competing for their niche (Challis and Hopwood 2003; van Wezel and McDowall 2011).

Fatty acids (FAs) are omnipresent molecules normally found bound to other compounds such as glycerol, sugars, or phosphate head groups to form lipids that are components of cell structures (e.g., membranes) and/or serve for energy storage. Free FAs have roles in host defenses of many multicellular organisms, including mammals, plants, mollusks, seaweeds, and amphibians against potential pathogenic or opportunistic microorganisms and could be biosynthesized de novo or released from lipids, typically by enzyme action (Desbois and Smith 2010; Chan and Vogel 2010). Free FAs are considered as very attractive antibacterial agents for various applications in medicine (especially for treating skin infections), agriculture, food preservation, and formulation of cosmetics or nutraceuticals, mainly because, in addition to a broad spectrum of activity, non-specific mode of action and lower probability toward development of inducible resistance than with conventional antibiotics, they are available from natural sources and generally regarded as safe and non-toxic for human (Drake et al. 2008; Desbois and Smith 2010). Furthermore, in the last decade, there is an increasing number of studies dealing with the reduction of biodiesel production costs by using less expensive feedstocks with high content of free FAs like non-edible oils, animal fats and oils, recycled or waste oils, and byproducts of the refining vegetable oils (Veljković et al. 2006).

Membrane lipid homeostasis is essential for bacterial survival and adaptation to different environmental conditions. Bacteria have evolved mechanisms to control the formation of new FAs and modify the structure of existing FAs, and these allow bacteria to adjust membrane viscosity to match environmental requirements (Zhang and Rock 2008). The ability of bacteria to modify their membrane composition in response to environmental changes, such as in temperature, osmolarity, salinity, and pH, was determined early in the study of bacterial lipid metabolism. FA biosynthesis in *Streptomyces* spp. relies exclusively on FAS type II that are discrete monofunctional enzymes contributing FAs not only to building membrane phospholipids but to storage triacylglycerides (Arabolaza et al. 2008; Gago et al. 2011). It is established that a number of functionally differentiated FAS variants have evolved by slight variations in the FAS pathways to produce a wide range of natural compounds, such as bioactive polyketides (PKS), in streptomycetes (Hertweck 2009; Gago et al. 2011; Florova et al. 2002). Having in mind the importance of the genus *Streptomyces*, the FA biosynthetic genes and their transcriptional control had received surprisingly little attention compared to other bacteria (Arabolaza et al. 2010; Gago et al. 2011). On the other hand, in-depth structural diversity of FA pool in these organisms has not been addressed up to date.

The present study describes the isolation, characterization, and identification of a novel *Streptomyces* strain designated as NP10 that produces considerable amounts of *n*- and branched-free FAs. In order to ascertain the possible physiological role of these metabolites, free FAs were assayed for antimicrobial activity against a panel of microorganisms and the effect on quorum sensing in *Pseudomonas aeruginosa*. The unique nature of NP10 strain was investigated with regards to high adaptability to harsh environmental conditions (temperature, pH, salt concentration, nutrients). Moreover, other *Streptomyces* strains were screened for their capability to produce free FAs. To the best of our knowledge, this is the first report on *Streptomyces* strain that could accumulate and excrete free FAs as a natural response to ecological competition and the first comprehensive assessment of FAs pool from an aerobic soil microorganism.

Materials and methods

Isolation, characterization, and maintenance of the bacterial isolate

A novel actinomycetes strain was isolated from a soil sample taken underneath a decaying wood from village Čumić, Central Serbia as previously described (Djokic et al. 2011). Genomic DNA from NP10 strain was extracted (Kapa Express Extract Kit, Kapa Biosystems, Wilmington, MA, USA) and 16S ribosomal DNA (rDNA) gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers 27f and 1492r (Lane 1991). The obtained PCR product was sequenced using Applied Biosystems 3130 Genetic Analyzer (Foster City, CA, USA). Sequences were analyzed and assembled by with SeqMan Pro software (DNASTAR Inc., Madison, WI, USA). The BLASTN program (NCBI, http://www.ncbi.nlm.nih.gov; Altschul et al. 1997) and the Seqmatch tool of the Ribosomal Database Project-II Release 9.4 (RDP-II; http;//rdp.cme.msu.edu; Cole et al. 2009) were used for sequences similarity searches. Alignment of NP10 strain sequence and homologous sequences taken from RDP was performed with Clustal W 2.0 algorithm (Larkin et al. 2007). Phylogenetic tree of strain NP10 was constructed by the maximum-likelihood algorithm using Jukes-Cantor distance correction and Bootstrap resampling method, all included in MEGA6 package (Tamura et al. 2013). The tree was rooted using 16S rDNA sequence of *Streptomyces lividans* NRRL B-12275^T as an out-group.

Strain NP10 was deposited at the Institute of Soil Science (Belgrade, Serbia) culture collection ISS WDCM375 under accession number ISS613, and the 16S rRNA gene sequence was deposited in GeneBank (JQ288108).

Spore suspension of *Streptomyces* sp. NP10 was prepared in 20 % glycerol (Kieser et al. 2000), maintained at −80 °C,

and used for the inoculation of cultures for further experiments. Starter culture was grown by inoculating spores (20 μL) into 100-mL flasks containing 20 mL vegetative medium (maltose 15 g L^{-1}, tryptic soy broth 8 g L^{-1}, yeast extract 4 g L^{-1}, $CaCO_3$ 2 g L^{-1}) and incubated during 48 h in a shaking incubator set at 30 °C and 180 rpm. These starter cultures were used for the inoculation of different media (0.4 %, *v*/*v*) in Erlenmeyer flasks containing coiled stainless steel wires for better aeration and incubated in the dark at 30 °C and 180 rpm in a shaking incubator. Cultures were also grown at temperature range of 4–42 °C and pH range of 2–10.

Cellulolytic activity of the isolate was assayed on the plates containing carboxymethyl cellulose (CMC) according to previously published method (Kasana et al. 2008) while hemolytic activity was assessed using commercial blood agar plates (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Preparation of crude and FAs enriched extracts from NP10 culture

Crude culture extracts of *Streptomyces* sp. NP10 were prepared by growing the strain in 400 mL of MSY medium (maltose 30 g L^{-1}, tryptic soy broth 8 g L^{-1}, yeast extract 4 g L^{-1}, $CaCO_3$ 2 g L^{-1}, $NaNO_3$ 3 g L^{-1}, $MnSO_4{\cdot}7H_2O$ 0.6 g L^{-1}, $ZnSO_4$ 0.005 g L^{-1}, $FeSO_4{\cdot}7H_2O$ 0.3 g L^{-1}, $CoCl_2{\cdot}7H_2O$ 5 mg L^{-1}) for 6 days at 30 °C shaking at 180 rpm.

Total cultures (mycelium and medium broth) were extracted with an equal volume of ethyl acetate or hexane/chloroform mixture (4:1, *v*/*v*) for the preparation of crude and FA-enriched extracts, respectively. Crude culture extracts were fractionated using flash chromatography that employed silica gel 60 (230–400 mesh; Merck, Darmstadt, Germany), while collected fractions were analyzed by thin-layer chromatography using aluminum-backed plates with a 0.25-mm silica layer (Kieselgel 60 F_{254}; Merck, Darmstadt, Germany) and *i*-16:0 as standard and by UV–vis spectral analysis. The following solvent system was used for fractionation: *n*-hexane and ethyl acetate (7:3, *v*/*v*, 100 mL), *n*-hexane and ethyl acetate (1:1, 100 mL), ethyl acetate and methanol (7:3, 100 mL), followed by ethyl acetate and methanol (1:1, 100 mL). Appropriate fractions were collected, organic phase was removed under reduced pressure, and fractions were weighted.

In vitro bioactivity assays

For in vitro assays, dried ethyl acetate extracts or the chromatographic fraction 11 (FR11) containing free fatty acids were weighted and redissolved in DMSO, allowing for the different concentrations/amounts to be used in the tests.

Antimicrobial testing was carried out using standard disc diffusion (200 μg per disc) and microdilution (Zgoda and Porter 2001) assays on a panel of organisms obtained from the American Type Culture Collection (ATCC). They included the following: *Micrococcus luteus* ATCC 379, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, *P. aeruginosa* PAO1 ATCC 27853, *Candida albicans* ATCC 10231, and *C. albicans* ATCC 10259. Strains of *Saccharomyces cerevisiae* FAV20 and FAS20 designed for detection of immunosuppressive activity (Skoko et al. 2005) were also included.

To test the effect of *Streptomyces* sp. NP10 extracts on pyocyanine production in *P. aeruginosa* PAO1, modified protocol of O'Loughlin et al. (2013) was used. *P. aeruginosa* PAO1 was grown in Kings A medium (1.5 % glycerol, 20 g L^{-1} peptone, 1.64 g L^{-1} $MgCl_2$, 10 g L^{-1} K_2SO_4) at 37 °C for 24 h. Overnight, *P. aeruginosa* PAO1 culture was diluted 1:1000 into a 5-mL Kings A medium and after addition of 50 or 100 μg mL^{-1} extracts from *Streptomyces* sp. NP10, 50 or 100 μL mL^{-1} overnight *Streptomyces* sp. NP10 culture or an equivalent amount of dimethyl sulfoxide, cultured for another 24 h at 37 °C with shaking. Culture aliquot (1 mL) was harvested at 14,000 rpm for 20 min and supernatant was analyzed for pyocyanin on a UV–vis spectrophotometer ultrospec 3300pro (Amersham Biosciences, Piscataway, NJ, USA) at 695 nm. All experiments were performed in triplicate and repeated at least three times.

In vitro cytotoxicity assay was performed on MRC5 and B12 cell line (human lung fibroblast and melanoma, obtained from ATCC) in Gibco® RPMI-1640 medium (Life Technologies Corporation, Carlsbad, CA, USA) supplemented with 100 μg mL^{-1} streptomycin, 100 U mL^{-1} penicillin, and 10 % fetal bovine serum (FBS). Both cell lines cells were treated with increasing concentrations (1 ng mL^{-1} to 1 mg mL^{-1}) of *Streptomyces* sp. NP10 crude or FAs containing extracts for 24 h, and cytotoxicity was determined using MTT reduction assay (Hansen et al. 1989). Hemolytic effect of the extracts on sheep erythrocytes was evaluated using the method described by Suthindhiran and Kannabiran (2009). Sheep red blood cells in 0.1 M phosphate buffered saline pH 7.4 (1 %*v*/*v*, Torlak, Belgrade, Serbia) were treated in hexaplicate with 1, 10, or 100 μg mL^{-1} of NP10 extracts at 37 °C for 1 h. Hemoglobin absorbance was measured at 405 nm on Labsystem Multiskan EX plate reader (MTX Lab Systems Inc., Vienna, VA, USA). The hemolysis percentage was calculated using the following equation: hemolysis (%)= $100[(Abs_{405\ nm}\ (\text{treated}) - Abs_{405\ nm}\ (\text{non-treated})/(Abs_{405\ nm}\ (0.1\ \%\ \text{Triton X-100 lysed}) - Abs_{405\ nm}\ (\text{non-treated})]$. Commercial *i*-16:0 (Sigma Aldrich, St. Louis, MO, USA) was included as control in bioactivity assays.

Gas chromatography–mass spectrometry (GC-MS)

The GC-MS analyses were performed in triplicate on a Hewlett–Packard 6890 N gas chromatograph equipped with a fused silica capillary column DB-5 (5 % phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 μm; Agilent Technologies, Santa Clara, CA, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 and 330 °C, respectively. The oven temperature was raised from 70 to 315 °C at a heating rate of 5 °C min^{-1} and then isothermally held for 10 min. Helium at 1.0 mL min^{-1} was used as a carrier gas. The samples, 5 μL of the corresponding solutions, were injected in a pulsed splitless mode (the flow was 1.5 mL min^{-1} for the first 0.5 min and then set to 1.0 mL min^{-1} throughout the remainder of the analysis). The mass selective detector was operated at the ionization energy of 70 eV, in the 35–650 amu range, with a scanning speed of 0.34 s. The percentage composition was computed from the GC peak areas without the use of correction factors.

Nuclear magnetic resonance (NMR)

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 400 spectrometer (Bruker Corporation, Fällanden, Switzerland) operating at 400 and 100 MHz, respectively. 2D experiments: ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single-quantum correlation spectroscopy (HSQC), and heteronuclear multiple-bond correlation spectroscopy (HMBC) were run on the same instrument with the usual pulse sequences. All NMR spectra were measured at 25 °C in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm (δ) and referenced to TMS (δ_H= 0 ppm) in ^{1}H NMR spectra, or to residual $CHCl_3$ (δ_H= 7.25 ppm) and $^{13}CDCl_3$ (δ_C=77 ppm) in heteronuclear 2D spectra. Scalar couplings are reported in hertz (Hz).

Liquid chromatography–time of flight mass spectrometry (LC-TOF/MS)

The LC-TOF/MS analysis was achieved on a Agilent 1200 Series HPLC instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler, Zorbax Eclipse Plus C18 column (150×4.6 mm, i.d.; 1.8 μm) and a diode-array detector, and coupled with a 6210 TOF LC/MS system of the same company. The mobile phase was a gradient prepared from 0.2 % formic acid in water (A) and acetonitrile (B), according to the following program: 0–1.5 min, 5 % B; 1.5–26 min 5–95 % B; 26–35 min 95 % B; 35–36 min 95–5 % B; 36–41 min 5 % B. ESI-MS spectra were recorded in the range *m/z* 100–3200 in positive ion mode, with 4000 V ion source potential and 140 V of fragmentor potential.

Qualitative analysis of fatty acid methyl esters (FAMEs)

Qualitative analysis of FAMEs was based on at least two of the following three means: positive matches of linear retention index (RI) values and mass spectra with those in the literature and GC co-injection with an authentic sample (Table 1). Authentic samples of methyl esters of FAs available in our laboratory (7:0–22:0, *i*-16:0, 11:1ω1, 16:1ω7c, 18:1ω9c, and 18:2ω6c) were prepared by synthesis performed in GC vials via addition of ethereal solution of CH_2N_2. Cyclopropane FAMEs standards were prepared from corresponding monoenoic FAMEs with CH_2N_2 in the presence of $Pd(PhCN)_2Cl_2$ as a catalyst (Gangadhar et al.1988). Epoxy FAMEs standards were obtained from corresponding mono- and dienoic FAMEs using *m*-chloroperoxybenzoic acid in $CHCl_3$ at room temperature (Aerts and Jacobs 2004). In order to prepare standards of *vicinal* dihydroxy FAME (9,10-*di*OH-18:0), the corresponding epoxy FAME was hydrolyzed in tetrahydrofuran:H_2O:0.5 % aq. $HClO_4$ (3:1:1) at room temperature (Moghaddam et al. 1996). Ethyl esters were synthesized by a Steglich procedure, utilizing *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine (Radulović et al. 2012).

Quantitative analysis of FAMEs

The quantification of FAMEs in chromatographic fractions and whole cell extracts was carried out by peak-area integration. Authentic standards of methyl esters of *i*-16:0, 16:0, 16:1ω7c, and 17:0cy9-10 were injected at seven different concentrations (1, 2.5, 10, 25, 100, 2500, and 1000 μg mL^{-1}) in order to build up seven-point GC-MS calibration curves for certain FAMEs class by plotting compound concentration versus peak area ($C=f(A)$). Each sample was analyzed for three consecutive runs.

Dimethyldisulfide (DMDS) derivatization

The samples of chromatographic fractions and whole cell extracts obtained after derivatization with CH_2N_2 were dissolved in DMDS (0.25 mL per mg of sample) and a solution (0.05 mL per mg of sample) of I_2 in diethyl ether (Et_2O; 60 mg mL^{-1}) was added. The mixture was stirred at room temperature overnight. Then Et_2O (5 mL per mg of sample) was added, and the obtained mixture was washed with 10 % aq. $Na_2S_2O_3$, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was taken up in fresh Et_2O (0.5 mL) and directly analyzed by GC-MS.

Table 1 Free FA composition of *Streptomyces* sp. NP10

RI[a]	Compound	Designation	Class	FR 11[b]	Method of identification[c]
1122	Methyl octanoate	8:0	N	0.022[d]	RI, MS, CoI
1186	Methyl 7-methyloctanoate	*i*-9:0	I	tr	RI, MS
1222	Methyl nonanoate	9:0	N	0.050	RI, MS, CoI
1287	Methyl 8-methylnonanoate	*i*-10:0	I	0.025	RI, MS
1323	Methyl decanoate	10:0	N	0.050	RI, MS, CoI
1388	Methyl 9-methyldecanoate	*i*-11:0	I	tr	RI, MS
1396	Methyl 8-methyldecanoate	*a*-11:0	A	tr	RI, MS
1404	Methyl 10-undecenoate	11:1ω1	U	0.026	RI, MS, CoI
1424	Methyl undecanoate	11:0	N	0.029	RI, MS, CoI
1488	Methyl 10-methylundecanoate	*i*-12:0	I	0.045	RI, MS
1524	Methyl dodecanoate	12:0	N	0.170	RI, MS, CoI
1588	Methyl 11-methyldodecanoate	*i*-13:0	I	0.060	RI, MS
1596	Methyl 10-methyldodecanoate	*a*-13:0	A	0.081	RI, MS
1598	Methyl (*Z*)-4-tridecenoate	13:1ω9c	U	0.033	MS
1624	Methyl tridecanoate	13:0	N	0.043	RI, MS, CoI
1688	Methyl 12-methyltridecanoate	*i*-14:0	I	0.906	RI, MS
1699	Methyl (*Z*)-9-tetradecenoate	14:1ω5c	U	0.026	RI, MS, DMDS
1724	Methyl tetradecanoate	14:0	N	0.189	RI, MS, CoI
1753	Ethyl 12-methyltridecanoate		E	0.035	RI, MS
1788	Methyl 13-methyltetradecanoate	*i*-15:0	I	0.499	RI, MS
1796	Methyl 12-methyltetradecanoate	*a*-15:0	A	1.114	RI, MS
1824	Methyl pentadecanoate	15:0	N	0.206	RI, MS, CoI
1853	Ethyl 13-methyltetradecanoate		E	0.033	RI, MS
1861	Ethyl 12-methyltetradecanoate		E	tr	RI, MS
1863	Methyl (*Z*)-14-methylpentadec-9-enoate	*i*-16:1ω6c	U	0.105	RI, MS, DMDS
1888	Methyl 14-methylpentadecanoate	*i*-16:0	I	1.390	RI, MS, CoI
1899	Methyl (*Z*)-9-hexadecenoate	16:1ω7c	U	0.292	RI, MS, CoI, DMDS
1924	Methyl hexadecanoate	16:0	N	1.264	RI, MS, CoI
1953	Ethyl 14-methylpentadecanoate		E	0.054	RI, MS, CoI
1966	Methyl 8-(2-(4-methylpentyl)cyclopropyl)octanoate	*i*-17:0cy9-10	CP	0.189	RI, MS
1971	Methyl (*Z*)-14-methylhexadec-9-enoate	*a*-17:1ω7c	U	tr	RI, MS, DMDS
1988	Methyl 15-methylhexadecanoate	*i*-17:0	I	0.301	RI, MS
1989	Ethyl hexadecanoate		E	tr	RI, MS, CoI
1996	Methyl 14-methylhexadecanoate	*a*-17:0	A	0.459	RI, MS
2002	Methyl 8-(2-hexylcyclopropyl)octanoate	17:0cy9-10	CP	0.372	RI, MS, CoI
2024	Methyl heptadecanoate	17:0	N	tr	RI, MS, CoI
2063	Methyl (*Z*)-16-methylheptadec-9-enoate	*i*-18:1ω8c	U	0.107	RI, MS, DMDS
2074	Methyl 8-(2-(4-methylhexyl)cyclopropyl)octanoate	*a*-18:0cy9-10	CP	0.087	RI, MS
2088	Methyl 16-methylheptadecanoate	*i*-18:0	I	0.053	RI, MS
2089	Methyl (*Z*,*Z*)-9,12-octadecadienoate	18:2ω6c	U	0.067	RI, MS, CoI
2099	Methyl (*Z*)-9-octadecenoate	18:1ω9c	U	0.181	RI, MS, CoI, DMDS
2124	Methyl octadecanoate	18:0	N	0.017	RI, MS, CoI
2171	Methyl (Z)-16-methyloctadec-9-enoate	*a*-19:1ω9c	U	tr	RI, MS
2188	Methyl 17-methyloctadecanoate	*i*-19:0	I	tr	RI, MS
2195	Ethyl octadecanoate		E	tr	RI, MS, CoI
2196	Methyl 16-methyloctadecanoate	*a*-19:0	A	0.024	RI, MS
2202	Methyl 8-(2-octylcyclopropyl)octanoate	19:0cy9-10	CP	0.038	RI, MS, CoI
2224	Methyl nonadecanoate	19:0	N	0.021	RI, MS, CoI
2288	Methyl 18-methylnonadecanoate	*i*-20:0	I	tr	RI, MS

Table 1 (continued)

RI[a]	Compound	Designation	Class	FR 11[b]	Method of identification[c]
2324	Methyl eicosanoate	20:0	N	0.022	RI, MS, CoI
2424	Methyl heneicosanoate	21:0	N	0.017	RI, MS, CoI
2534	Methyl docosanoate	22:0	N	0.018	RI, MS, CoI
2624	Methyl tricosanoate	23:0	N	tr	RI, MS
2724	Methyl tetracosanoate	24:0	N	0.017	RI, MS
2824	Methyl pentacosanoate	25:0	N	tr	RI, MS
2924	Methyl hexacosanoate	26:0	N	tr	RI, MS
3124	Methyl octacosanoate	28:0	N	tr	RI, MS
	Total			8.899 (56)	
	Saturated fatty acid methyl esters				
	Normal chain (N)			2.134 (20)[e]	
	Even-numbered			1.768 (11)	
	Odd-numbered			0.366 (9)	
	Iso (I)			3.279(12)	
	Even-numbered			2.419 (6)	
	Odd-numbered			0.860 (6)	
	Anteiso (A)			1.678 (5)	
	Even-numbered			n.d.	
	Odd-numbered			1.678 (5)	
	Unsaturated fatty acid methyl esters (U)			0.817 (10)	
	Normal chain			0.625 (6)	
	iso			0.192(2)	
	anteiso			tr (2)	
	Cyclopropane fatty acid methyl esters (CP)			0.599 (3)	
	Saturated fatty acid ethyl esters (E)			0.122 (6)	

tr trace (<0.007 μg mL^{-1}), *n.d.* not detected

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

[b] FR11—a chromatographic fraction (no. 11) of the total solvent extract containing FAs

[c] 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 patterns of the corresponding DMDS adducts

[d] Concentration is expressed as μg per mL of broth

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

Alkaline transesterification using solution of sodium methoxide in methanol (CH_3ONa/CH_3OH)

A solution of CH_3ONa was prepared by dissolving 50 mg of metallic sodium in anhydrous CH_3OH (5 mL), which was then cooled to room temperature. A portion of the whole cell extract (*ca*. 20 mg) dissolved in CH_3OH was added with stirring to this solution, brought to reflux and quenched with excess ice-water. This was followed by immediate extraction of the reaction mixture with Et_2O, the organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The obtained oily residue was dissolved in Et_2O (0.5 mL) and analyzed by GC-MS.

Results

Description of the soil isolate *Streptomyces* sp. NP10

An aerobic, rapidly growing, and sporulating (24–30 h at 30 °C) bacterium was isolated from the soil sample taken underneath a decaying wood (village Čumić, Serbia) and designated NP10 (Fig. 1). Surface-grown culture developed light yellow oily droplets on the surface of mature white spore chains (Fig. 1a), while no exogenous pigment was developed even under prolonged incubation. On the basis on 16S rDNA sequence analysis, NP10 was affiliated with genus *Streptomyces* (Fig. 1b). Based on the database search (Ribosomal Database Project, http://rdp.cme.msu.edu/

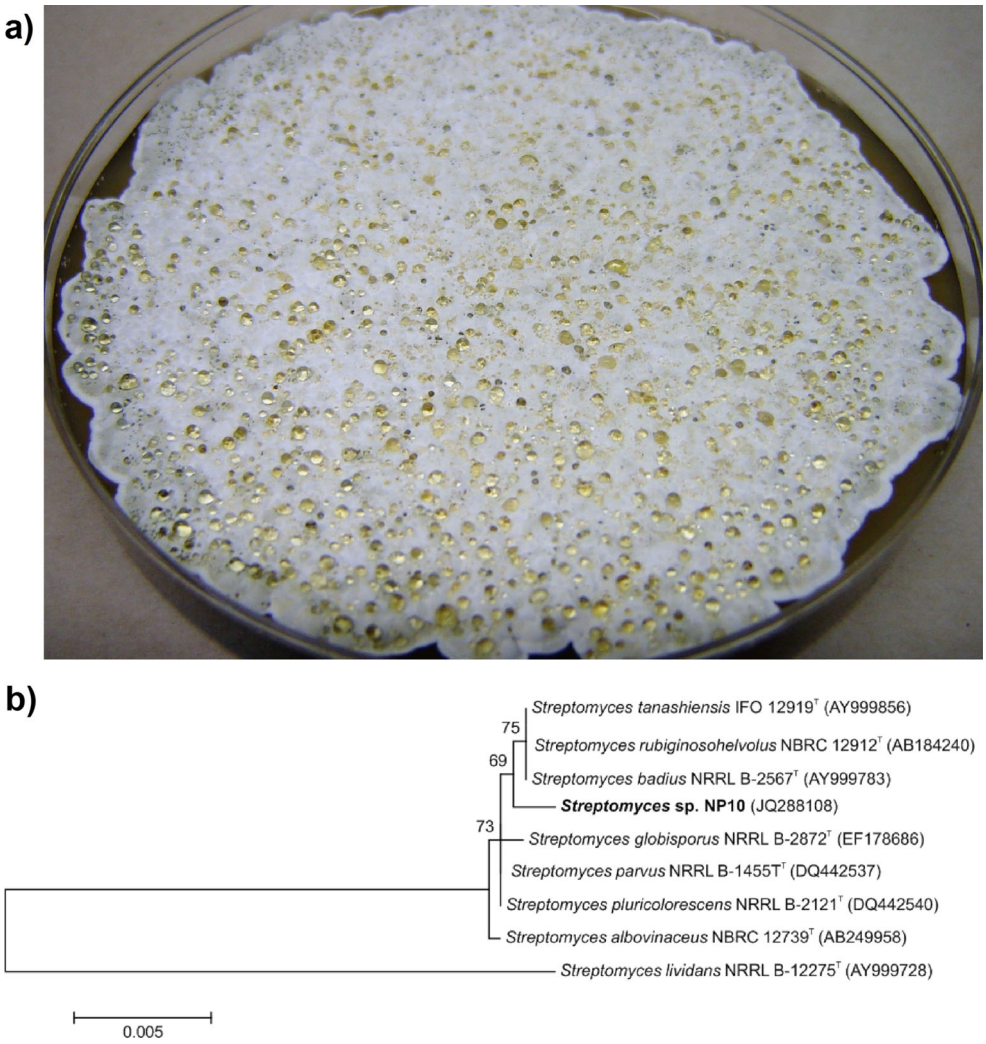


Fig. 1 **a** The isolate NP10 with white aerial mycelium and white spores developing oily-like droplets on the surface of the culture grown on casein starch agar. **b** Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationship of isolate *Streptomyces* sp. NP10 (*designated in bold*) and closely related strains, using *Streptomyces lividans* NRRL B-12275^T as an out-group. Bootstrap values at branch points are expressed as a percentage of 1000 replications. GeneBank accession numbers are in *brackets*. The *scale bar* represents 0.005 substitutions per nucleotide position

index.jsp) of rDNA sequence the largest overlap (with probability coefficient of 0.997) was with *Streptomyces badius* NRRL B-2567^T and with *Streptomyces rubiginosohelvolus* NBRC 12912^T. These results are in accordance with the search results of BLAST NCBI nucleotide collection (http://blast.ncbi.nlm.nih.gov/Blast.cgi). After bootstrapping, the phylogenetic tree of *Streptomyces* sp. NP10 was clustered with *S. badius*, *S. rubiginosohelvolus*, and *Streptomyces tanashiensis* (Fig. 1b).

Streptomyces sp. NP10 utilized a wide range of sugars including glucose, maltose, mannitol, glycerol, xylose and could not utilize sucrose as a sole source of carbon. Tests for starch, gelatin, and urea hydrolysis showed positive results, but nitrate reduction, H_2S, and indole production showed negative results. Biochemical tests revealed the presence of DNAse and hemolysin in this strain (Table S1 in the Supplementary Material). High cellulolytic and hemolytic activity was also detected in the solid medium (Fig. S1a, b in the Supplementary Material).

The strain has a very high growth rate and a short life cycle for a streptomycete. The optimum growth temperature was 28–30 °C, while surprisingly this strain could grow and sporulate well at 4–8 °C (10–14 days period required from spore germination to formation of colonies with mature spores) and could also grow at 37 °C, but not at 42 °C. *Streptomyces* sp. NP10 required no NaCl for growth, while it showed halotolerance of up to 12 % NaCl in the medium (Fig. S1c in the Supplementary Material). The optimum pH for *Streptomyces* sp. NP10 growth was 6–7, while it could also grow slower at pH 9. It can be concluded that *Streptomyces* sp. NP10 strain is remarkably tolerant to osmotic, cold and heat, and pH stress compared with other streptomycetes. In addition, cellulolytic activity makes it biotechnologically relevant.

Crude culture extracts of this strain showed antimicrobial activity in disc-diffusion assay (200 μg per disc) against *S. cerevisiae* FAV20, *S. cerevisiae* FAS20, *C. albicans*, *E. faecalis*, *S. aureus*, *B. subtilis*, and *M. luteus* (Fig. S2a in the Supplementary Material), while mild cytotoxic effect

against human fibroblasts and melanoma cell lines was exhibited only at high concentration of 1 mg mL^{-1} (Fig. S2b in the Supplementary Material).

Chemical characterization of antimicrobial fractions of *Streptomyces* sp. NP10 culture extract

Bioassay-guided fractionation of the ethyl acetate whole culture extract of the new *Streptomyces* strain NP10 pointed to fraction 11 (FR 11), eluted with a mixture of hexane/ethyl acetate (1:1) from the SiO_2 column, as active against *C. albicans* and *E. faecalis*. LC-TOF/MS analysis revealed that this fraction represented a mixture of several related compounds dominated by component(s) for which this high-resolution mass spectrometry technique predicted an empirical formula of $C_{16}H_{31}O_2$ for $[M–H]^-$ ion (at *m/z* 255.2329) indicating that it was a free saturated FA with 16 carbon atoms (Mohn et al. 2009). Moreover, the presence of a carboxylic group was confirmed by the signal at δ 180.15 ppm in ^{13}C NMR spectrum (Fig. 2b). The triplet at δ 2.345 ppm (J= 7.5 Hz) and quintet at δ 1.630 ppm (J=7.5 Hz) were also readily assigned to methylene protons in α- and β-position to the carboxyl acid group based on chemical shift value, splitting pattern, and the value of coupling constant (Fig. 2a). HSQC spectrum pointed to the existence of four different types of (terminal) methyl groups (Fig. 2c). Although the signals of these methyl groups overlapped in ^1H NMR spectrum (Fig. 2a), the doublet at δ 0.861 ppm (J=6.6 Hz) coupled with the septet at δ 1.513 ppm (J=6.6 Hz) could be straightforwardly assigned to an isopropyl spin system (Vyssotski et al. 2012). This was further confirmed by appropriate cross-peak correlations in $^1H–^1H$ COSY and HMBC spectra. Protons of methyl group that resonated at 0.880 ppm showed connectivity in HMBC spectrum with two methylene carbons (DEPT experiment), at δ 22.714 and 31.949 ppm, suggesting the existence of "CH_3-CH_2-CH_2-" structural moiety (Bengsch et al. 1986). Assignments of certain structural fragments of compounds from FR11 to appropriate signals in ^1H- and ^{13}C-NMR spectra, based on 2D homo- and heteronuclear-NMR experiments and available literature data, are shown in Fig. 2.

A portion of FR11 was then treated with an ethereal solution of CH_2N_2 in order to convert these free FAs to corresponding methyl esters, thus allowing their further qualitative and quantitative analyses by GC-MS. The GC-MS analysis enabled detection and identification of more than 55 components (Table 1, Fig. 3a) belonging to methyl (or ethyl) esters of normal and branched (*iso*- or/and *anteiso*-)-chain saturated, unsaturated, and cyclopropane FAs. This rather diverse and complex free FA profile of *Streptomyces* sp. NP10 was dominated by saturated *iso*- and *anteiso*-FAs with *i*-14:0 (0.906 μg mL^{-1}), *a*-15:0 (1.114 μg mL^{-1}), and *i*-16:0 (1.390 μg mL^{-1}) as the most abundant ones. These branched chain FAs were followed by normal chain isomers among which the highest content had 16:0 with 1.264 μg mL^{-1} that was ranked second in the overall composition.

In vitro activity of the free FAs and the extract of *Streptomyces* sp. NP10 culture

Although the crude *Streptomyces* sp. NP10 culture extract and FR11 (FAs containing fraction) exhibited antibacterial effects in disc diffusion assays, minimum inhibitory concentrations (MIC) in liquid cultures were rather high and comparable to that of commercial *i*-16:0. MICs of FAs extract were 500 μg mL^{-1} for *C. albicans* and *E. fecalis* while other strains were completely inhibited only at concentrations higher than 1 mg mL^{-1}. Interestingly, although no effect on the growth of *P. aeruginosa* PAO1 was observed at tested concentrations, an obvious lack of green color typical for untreated *P. aeruginosa* PAO1 cultures was noticed. This suggested that *Streptomyces* sp. NP10 FAs extracts had the ability to modulate pyocyanin production in *P. aeruginosa* PAO1 so this activity was examined further. Indeed, *Streptomyces* sp. NP10 crude extract, as well as FAs extracts reduced pyocyanin production in *P. aeruginosa* PAO1 strain between 10 and 30 % (Fig. 4a). The most pronounced effect had FAs extract at 100 μg mL^{-1} while live cells of *Streptomyces* sp. NP10 caused stimulatory effect on pyocyanin production (Fig. 4a). Therefore, it is possible that FAs produced by strain *Streptomyces* sp. NP10 have ecophysiological role in interspecies signaling and communication.

Streptomyces sp. NP10 crude culture extract caused 25 % hemolysis at 100 μg mL^{-1}, while FAs extracts as well as *i*-16:0 exhibited almost no hemolytic activity at same concentration (Fig. 4b). Hemolytic effect of the crude extracts can be due to the presence of other compounds. Furthermore, FAs exhibited only mild cytotoxicity at very high concentrations of 1 mg mL^{-1} (results not shown) which is in a good agreement with literature data that usually report stimulatory and protective effects of different FAs at lower concentrations (Mei et al. 2011). Saturated and unsaturated FAs differentially regulate apoptosis in various experimental systems in which saturated FAs were determined to be the more toxic lipid species (Ricchi et al. 2009; Mei et al. 2011).

Bound FA profile of *Streptomyces* sp. NP10

In bacterial cells, FAs occur mainly in the cell membranes as the acyl constituents of phospholipids (Kaneda 1991), although they could be occasionally found in the free form as minor components of neutral membrane lipids. Cellular (bound) FA analysis is regarded as a very useful and indispensable method, both in taxonomic studies and in identification of new bacterial species (Vandamme et al. 1996). Therefore, the bound FAs of new *Streptomyces* strain NP10 were also converted to methyl esters by alkaline

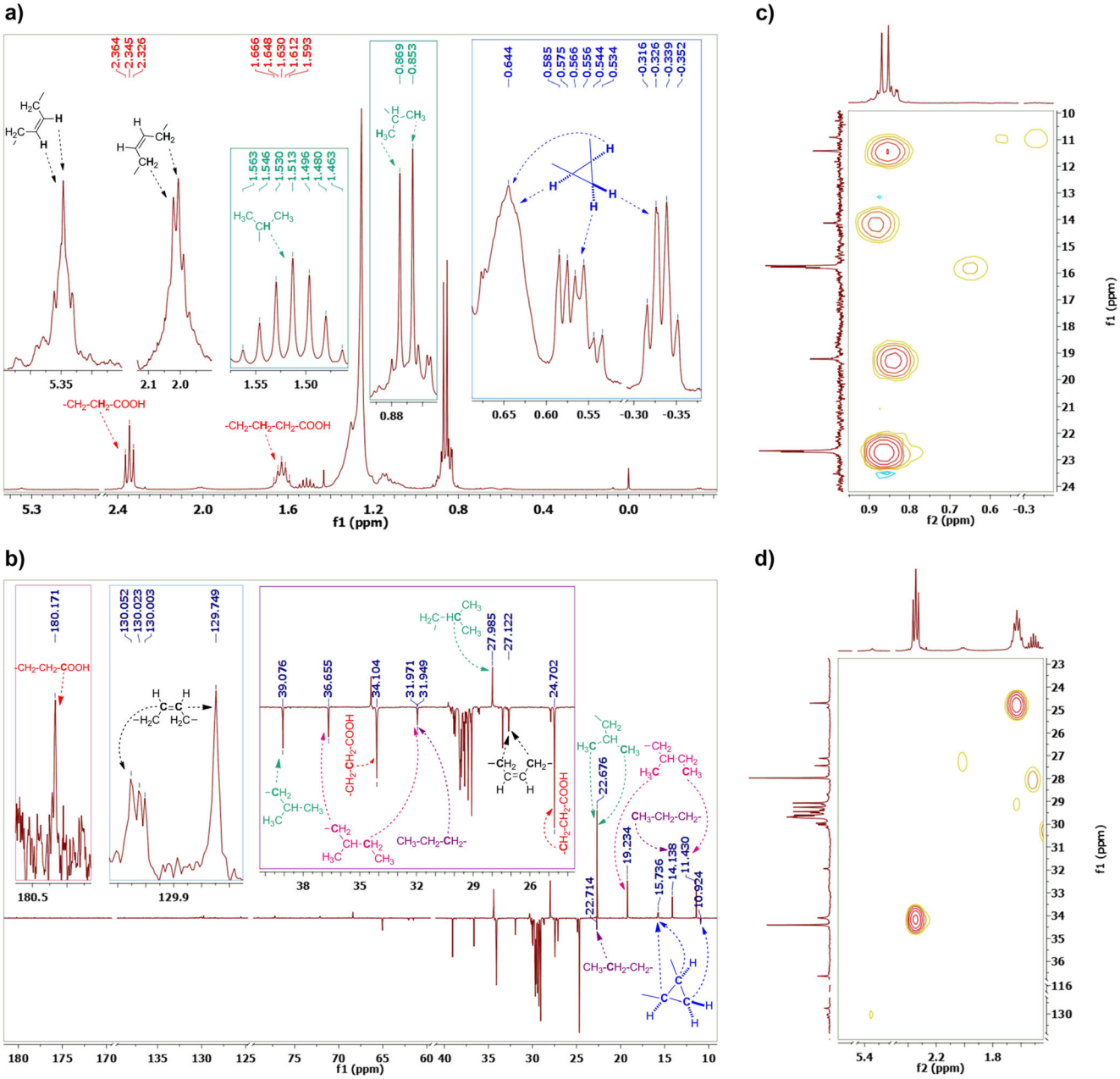


Fig. 2 Distinguishing structural fragments inferred from characteristic signals in **a** ^{1}H NMR, **b** ^{13}C NMR, and **c**, **d** HSQC spectrum of fraction 11

transesterification (CH_3ONa/CH_3OH; Radulović et al. 2012) and analyzed by GC-MS. Forty-five FAMEs were identified and they are listed in Table S2 in the Supplementary Material. Bound FA profile, like the free FA ones, was dominated by *i*-14:0 (11.2 %), *a*-15:0 (16.3 %), *i*-16:0 (17.7 %), and 16:0 (14.3 %) (Fig. 5a, b).

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, Fig. 3) and possessing analogous mass spectra. Full details of the structural elucidation are presented in Supplementary Material. The identification of saturated normal chain and branched (*iso*- and *anteiso*-) FAMEs was based on a combination of data coming from their mass spectra (Dickschat et al. 2011; Boon et al. 1977), NMR data (Fig. 2, Biermann and Metzger 2004) and gas chromatographic retention behavior (Fig. 3, Radulović et al. 2012). In addition to the mentioned

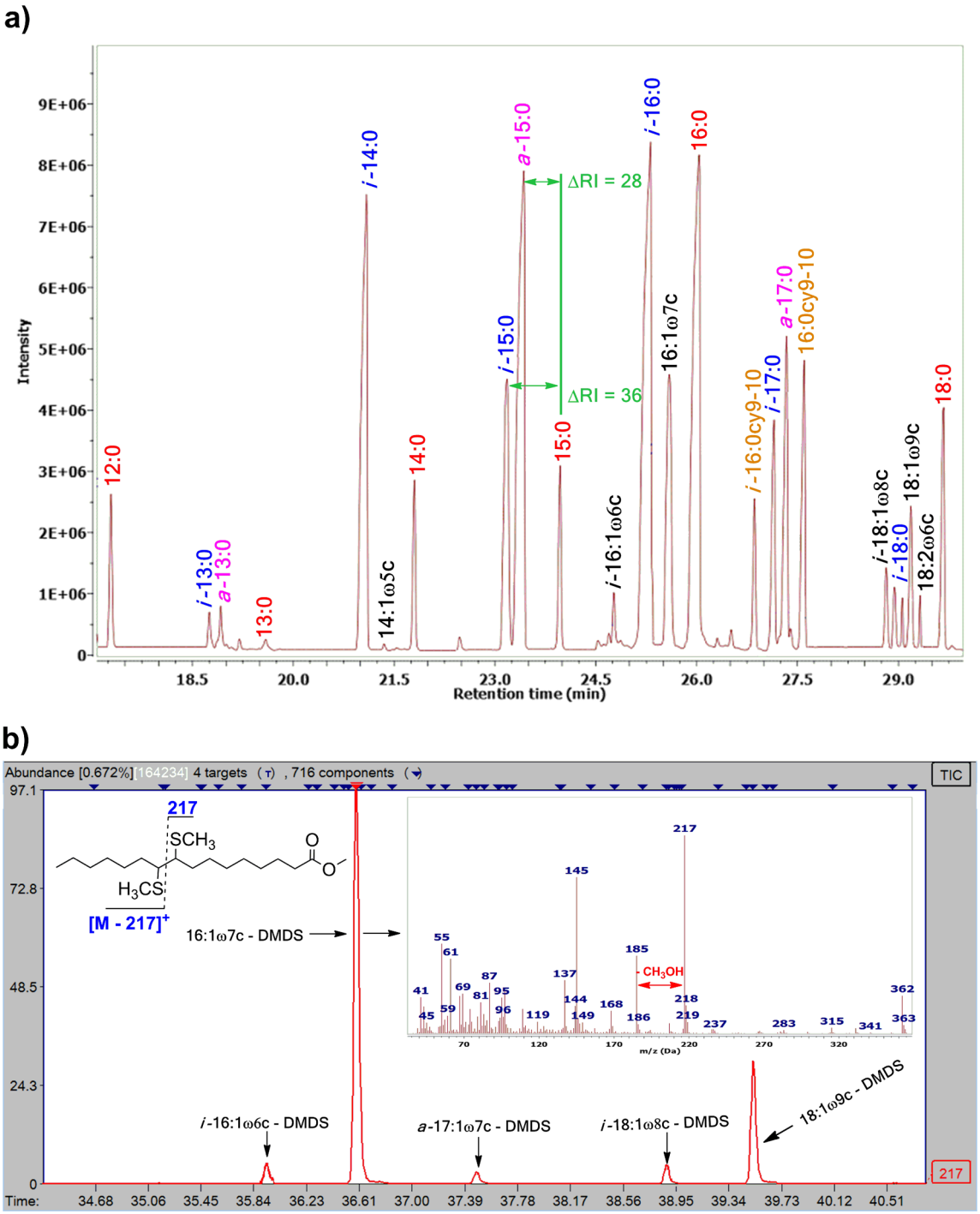


Fig. 3 **a** Total ion chromatogram of fraction 11 of *Streptomyces* sp. NP10 culture extract derivatized with CH_2N_2. **b** Total ion chromatogram after derivatization with DMDS with selected ion currant at *m/z* 217, mass spectrum and characteristic fragmentation pattern of DMDS adduct of 16:1ω7c

spectral and retention data, the structure of monounsaturated normal and branched FAMEs, more specifically the double bond position, was inferred from the MS fragmentation patterns of the corresponding DMDS adducts (Fig. 3, Rontani et al. 2009; Dickschat et al. 2005). The *cis* configuration of the double bonds in all detected monoenoic FAs was determined from the absolute value of the ^{13}C chemical shifts of allylic carbons (Santos and Graça 2014). The identification was corroborated wherever possible by a subsequent GC-MS analysis of authentic standards.

In order to clearly corroborate the presence of the cyclopropyl group in a number of detected FAMEs, as well

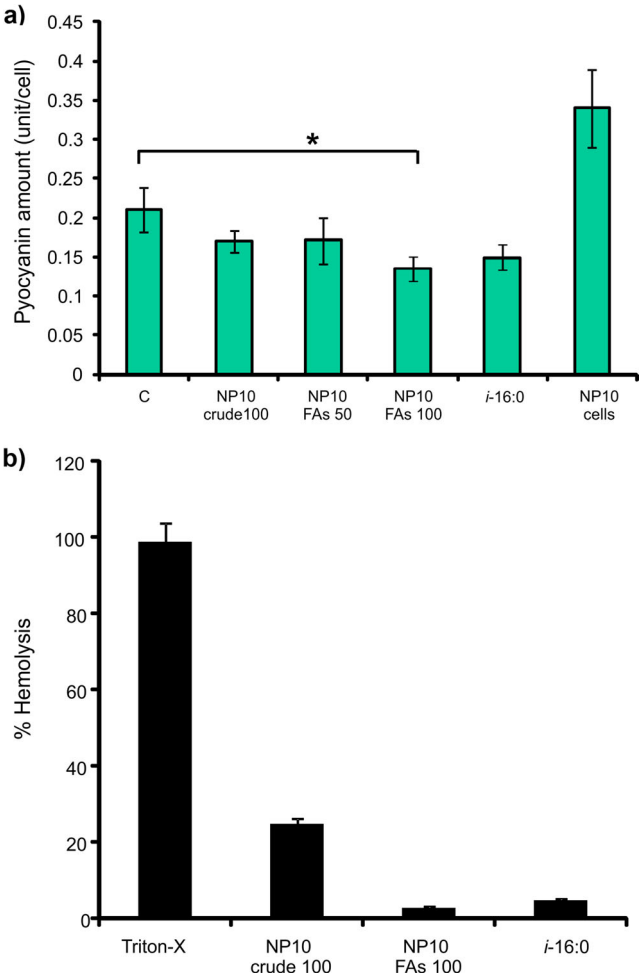


Fig. 4 In vitro activity of *Streptomyces* sp. NP10 extracts **a** on pyocyanine production in *Pseudomonas aeruginosa* PAO1 and **b** hemolytic effect. Crude culture extracts and *i*-16:0 acid standard were added at 100 μg mL^{-1}, while FAs extracts were added at concentrations 50 and 100 μg mL^{-1}. * signifies statistically relevant difference

as to assign its stereochemistry, the ^{1}H NMR spectrum, as well as other 1D and 2D NMR spectra, of non-derivatized FR11 were once closely inspected. Three high-field signals in the ^{1}H NMR spectrum, mutually coupled in homonuclear 2D spectra, were 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 positions 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.

Optimization of conditions for production of free FAs by *Streptomyces* sp. NP10

Firstly, different solvents were examined in order to optimize the extraction of free FAs produced by the strain *Streptomyces* sp. NP10. The mixture of hexane/$CHCl_3$ (4:1) was found to be the most suitable, so it was used for as the extraction medium in all further experiments (Fig. S4a, b in the Supplementary Material). In order to determine whether this strain accumulates or excretes these metabolites, mycelium and culture media were separated by filtration and extracted independently. The analysis revealed that significantly higher amounts of free FAs were present in the medium than in the mycelium (Fig. 5c, d). Then, time dependence of the production of free FAs by strain *Streptomyces* sp. NP10 was monitored and the maximal production (amount excreted to culture media) was observed at the sixth day of cultivation (Fig. 5e, f). In all previously described experiments, the strain was cultured on a complex medium rich in maltose (designated as MSY), and, finally, the influence of different growth media (maltose-soy flower (MSY), defined minimal R2m and minimal supplemented with yeast extract R2YE, respectively, media compositions are given in the Supplementary Material) on the production of free FAs, was studied. It was found that the cultivation on a complex medium rich in maltose (designated as MSY) as the carbon source was especially favorable for free FA production (Fig. S5a, b in the Supplementary Material).

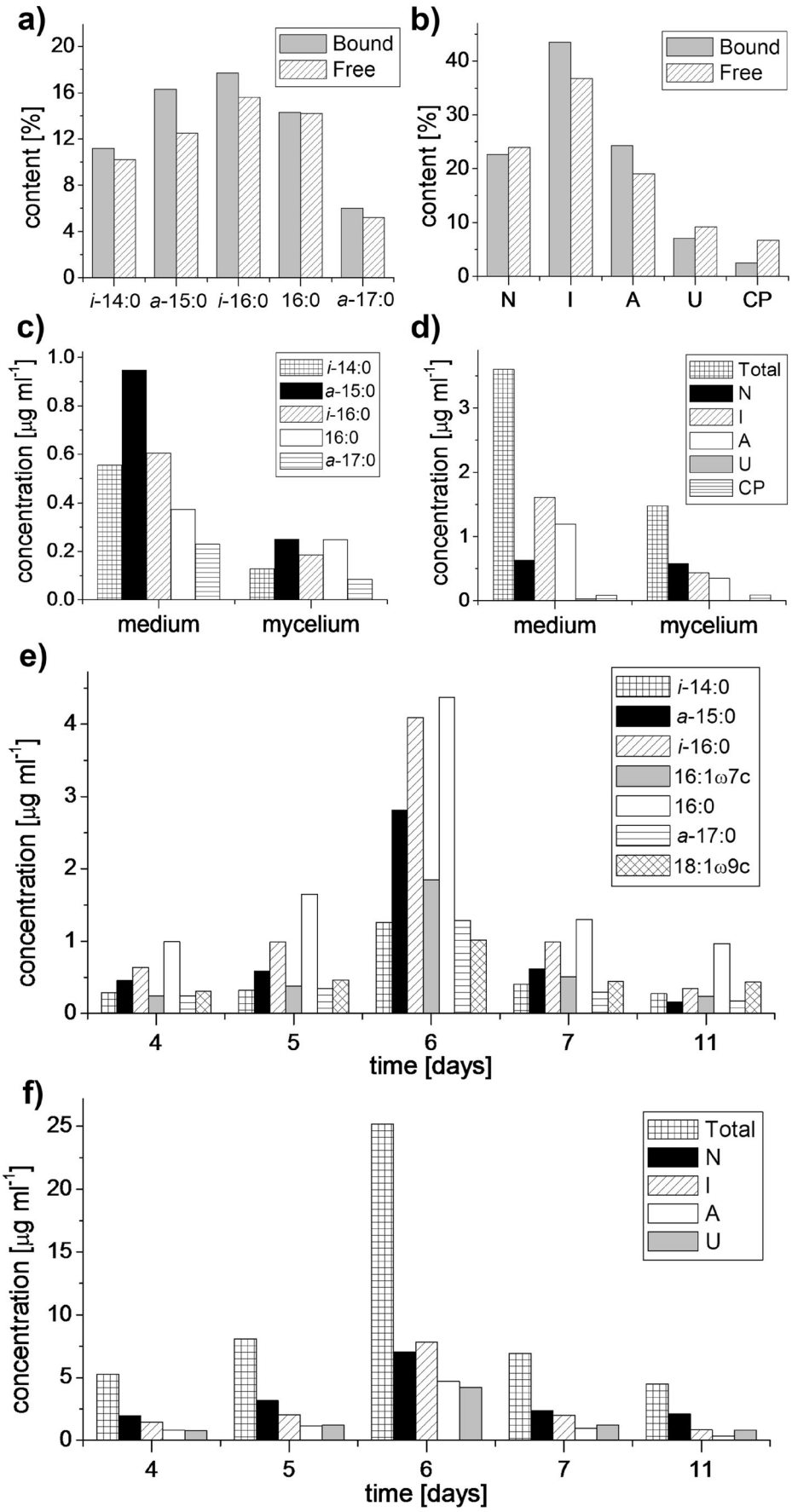


Fig. 5 Bound and free FA profiles of *Streptomyces* sp. NP10: **a** distribution of the major FAs and **b** certain classes of FAs. **c** Distribution of major free FAs in *Streptomyces* sp. NP10 culture between medium and mycelium and **d** distribution of certain classes of FAs. Time dependence of production and **e** distribution of main free FAs by *Streptomyces* sp. NP10 and **f** distribution of certain classes of FAs. *N* normal chain, *I iso*, *A anteiso*, *U* unsaturated, and *CP* cyclopropane FAs

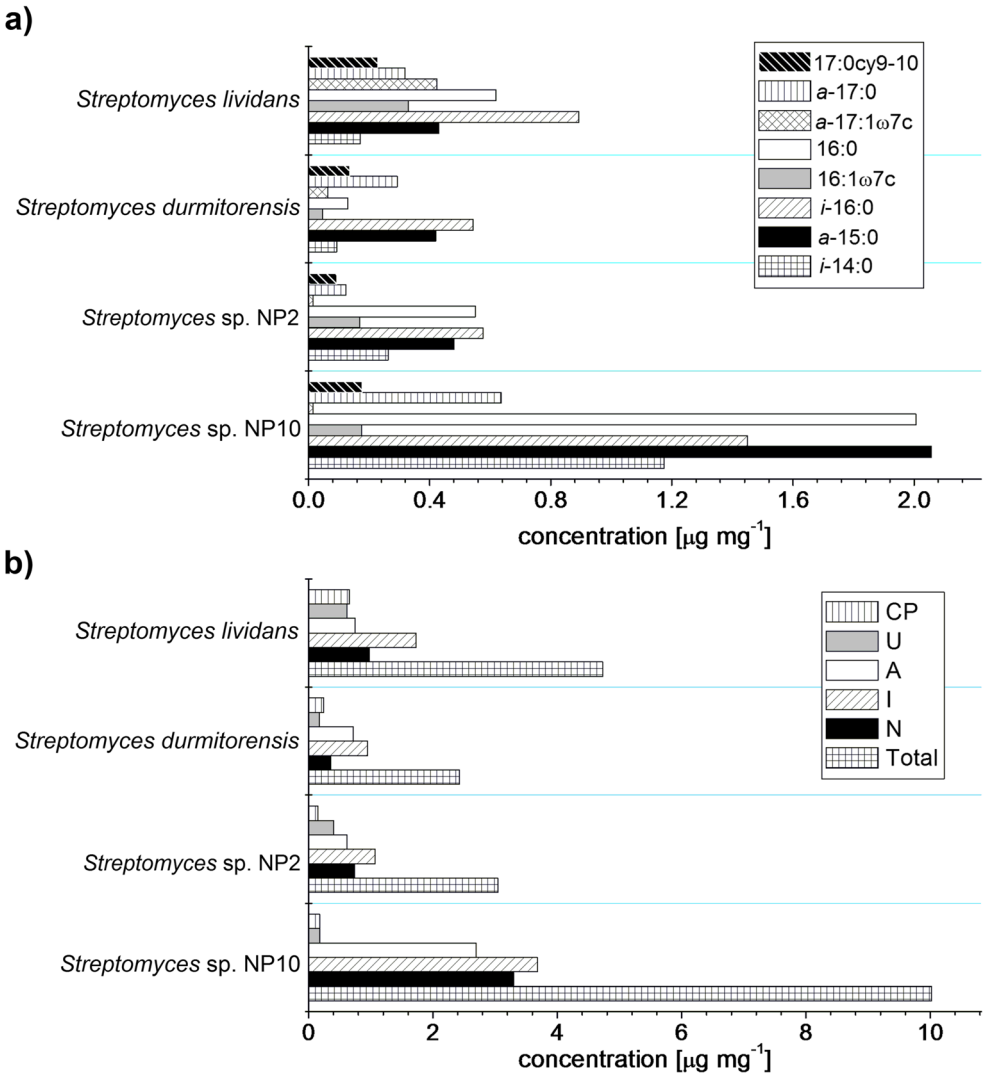


Fig. 6 Free fatty acid profiles of four different *Streptomyces* strains. **a** Distribution of main FAs. **b** Distribution of certain classes of FAs. *N* normal chain, *I iso*, *A anteiso*, *U* unsaturated, and *CP* cyclopropane FAs

Furthermore, growing on this medium, the strain was able to produce free FA even at 4 °C (Fig. S5a, b in the Supplementary Material). Cultivation at this low temperature resulted in a decrease of the total amount of produced free FAs, especially those of *iso*- and *anteiso*-chain. Thus, free FA profile at 4 °C was dominated with 16:0 and 18:0, and intriguingly, *a*-17:0 was detected only in trace amounts (Fig. S5a, b in the Supplementary Material).

Taxonomic significance of free FAs

Previous findings that free FAs could be found in small amounts in cell membranes of some other *Streptomyces* sp. (Metz et al. 1988; Hoischen et al. 1997) led us to examine if this increased production and secretion of free FAs is a unique feature of the particular strain *Streptomyces* sp. NP10 or it is perhaps a characteristic of all members of *Streptomyces* genus previously unpublished. Thus, we studied whether three other *Streptomyces* species available from our microbiological collection (both laboratory control strains and soil isolates) have the ability to produce free FAs. The analysis showed that all assayed strains: *S. lividans* NRRL B-12275, *Streptomyces durmitorensis* MS405, and *Streptomyces* sp. NP2-ISS618 synthesized free FAs and that their free FAs profiles (dominated by *a*-15:0 and *i*-16:0) were similar to that described for *Streptomyces* sp. NP10 (Fig. 6a, b, Table S3 in the Supplementary Material). The most notable differences were in the increased amount of unsaturated FAs synthesized (especially *i*-16:1ω6c, 16:1ω7c, and *a*-17:1ω7c) by *S. lividans* and of cyclopropane FAs (*i*-17:0cy9-10 and 17:0cy9-10) found in *Streptomyces* sp. NP2, as well as in the slightly decreased production of normal chain FAs (particularly 16:0) by the strain *S. durmitorensis* MS405 (Fig. 6a, b). Moreover, the total amount of produced free FAs varied among the analyzed strains from 2.427 μg mg^{-1} of dry mycelium in the case of *S. durmitorensis* to 10.021 μg mg^{-1} in the case of *Streptomyces* sp. NP10.

Table 2 Structure, designation, and occurrence in the nature of unusual FAs found in *Streptomyces* sp. NP10

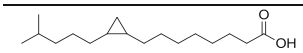
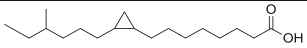
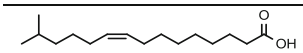
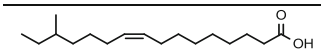
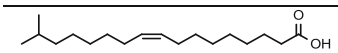
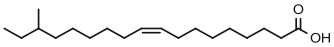
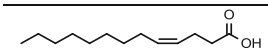
Fatty acid structure	Designation	Occurrence in the nature
	*i*-17:0cy9-10	Not found
	*a*-18:0cy9-10	Not found
	*i*-16:1ω6c	-Lipids of surface sediments from Corner Inlet, Victoria[a] -Cellular lipids of methanotrophic soil column[b] -Cellular fatty acid of *Streptomyces griseus*[c] - Cellular lipids of biofilter sample of the animal rendering plant[d, e] - Cellular lipids of the sulfate-reducing bacteria *Desulfosarcina variabilis*[e, f] - In free form in *Streptomyces* sp. A251[g]
	*a*-17:1ω7c	- Cellular lipids of the sulfate-reducing bacteria *Desulfovibvio desulfuvicans*[e, h] - Cellular lipids of the *Legionella micdadei* and *L. micdadei* Bari 2/158[e, i] - Cellular fatty acid of the sponge *Agelas conifera* from the Colombian Caribbean[e, j] -Cellular fatty acid of *Streptomyces griseus*[c] - Cellular fatty acid of the endemic fresh-water sponges *Baiealospongia baeillifera* and *Baicalospongia intermedia* (Lake Baikal)[e, k] - Cellular fatty acid of the endemic sponge *Lubomirskia baicalensis* (Lake Baikal) and its amphipod crustacean parasite *Brandtia* (*Spinacanthus*) *parasitica*[e, l] - In free form and as component of triacylglycerols of the leaf beetle *Chrysomela vigintipunctata* (Scopoli)[e, m] - Cellular lipids of the sulfate-reducing bacteria *Desulfosarcina variabilis*[e, n] - Lipids in sediment samples from the Lusatian mining district[e, o]
	*i*-18:1ω8c	- Cellular lipids of the sponge *Hymeniacidon sanguinea* from the Black Sea[p] - In total lipids from the green above-ground parts of alpine plant *Primula macrophylla*[e, q] - Cellular lipids of the microcosms samples freshly obtained sediment of a monitoring well from a petroleum hydrocarbon-contaminated aquifer[e, r]

Table 2 (continued)

Fatty acid structure	Designation	Occurrence in the nature
	*a*-19:1ω9c	- *Hesperopeuce mertensiana* (*Pinaceae*) seed lipids[s]
	13:1ω9c	-Not found in any bacteria species -In total lipids of leaves of *Salvia nemorosa*[t] - In total lipids of sericea lespedeza and bermudagrass hays [u]

[a] Nichols et al. 1985
[b] Nichols et al. 1986
[c] Suutari and Laakso 1993
[d] Knief et al. 2003
[e] Geometry of double bond was not specified
[f] Rütters et al. 2001
[g] Zheng et al. 2010
[h] Taylor and Parkes 1983
[i] Moss and Lambert-Fair 1990
[j] Duque et al. 1993
[k] Dembitsky et al. 1993
[l] Dembitsky et al. 1994
[m] Nikolova et al. 2000
[n] Rütters et al. 2001
[o] Poerschmann et al. 2012
[p] Christie et al. 1994
[q] Tsydendambaev et al. 2004
[r] Pelz et al. 2001
[s] Destaillats et al. 2002
[t] Agar et al. 2008
[u] Lee et al. 2012

Discussion

Although various *Streptomyces* spp. have been proven to be a prolific source of antibiotics and other useful metabolites, their involvement in the turnover of organic matter and xenobiotic compounds is only gaining more attention, which makes continuous search and isolation for novel species of this genus highly relevant. NP10 isolate reported in this study encompasses quite a few desirable traits that make it suitable for further biotechnological applications such as biofuels production (cellulolytic activity accompanied with FA accumulation in the medium). Cellulolytic *Streptomyces* strains have been encountered in the past while, only recently, a phylogenetically linked ability to degrade lignocellulose was described in *Streptomyces* associated with herbivorous insects (Book et al. 2014). On the other hand, hemolytic activity is usually not associated with *Streptomyces* spp., with some rare examples reported (Suthindhiran and Kannabiran 2009), while halotolerance is usually associated with *Streptomyces* isolated from desert soils or salty waters as a consequence of their adaptation to the saline environment (Thumar et al. 2010; Bhave et al. 2013). Halotolerant and alkaliphilic *S. aburaviensis* was isolated from the saline desert of Kutch in India; however, it required 5–10 % NaCl in the medium and pH 9 for the optimal growth (Thumar et al. 2010). Under varying cultivation conditions (incubation time, nutritive medium, temperature, etc.), *Streptomyces* NP10 was found to produce and excrete into the growth medium considerably large amounts of free long-chain FAs (C_7–C_{28}). The maximum production of free FAs by this strain should be expected

at the sixth day of cultivation in a complex medium rich in maltose at 28–30 °C. A detailed lipidomics study (consisting of a chromatographic isolation, NMR measurements, derivatizations, chemical transformations, and GC-MS co-injections), that followed, enabled the identification of over 50 different FAs of *n*-, *iso*-, and *anteiso*-chains including both saturated, unsaturated, and cyclopropane acids, among which *i*-14:0, *a*-15:0, *i*-16:0, and 16:0 were found to be the most abundant ones.

The qualitative and quantitative composition of the total cellular FAs (Fig. 5a, b) of this novel isolate was consistent with its affiliation to the genus *Streptomyces* where most species contained *iso*- and *anteiso*-branched chain FAs (primarily *a*-15:0 and *i*-16:0) as the major bound FAs (Saddler et al. 1987). The main difference in bound and free FA profiles of this new strain was the occurrence of oxygenated FAs: 18:0ep9-10c, 18:0*di*-ep9-10:12-13 (two isomers), and 9,10-*di*-OH-19:1 (two isomers) as minor component of total cellular FAs (Table S-2 in the Supplementary Material). To the best of our knowledge, the identified epoxy FAs have not been previously found as components of any microorganism. It has been debated that some epoxy and dihydroxy FAs reported from various organisms may be artifacts arisen from either spontaneous or enzymatic (ep)oxidation (and hydrolysis) during sample preparation (Bernard 2014). Generally, streptomycetes, with the exception of some fast-growing thermophilic strains, grow very slowly even at their optimal temperature (usually 28 to 30 °C; Chen and Qin 2011). In view of this, the observed ability of this novel *Streptomyces* strain to have noticeable growth rate at low temperature (4 °C) seems rather remarkable. This atypical feature could be possibly attributed to the very high content of *iso*- and *anteiso*-FAs (43.5 and 24.3 %, respectively) in its membrane since it is well established that a major aspect of the cryotolerant physiology of some bacterial species, such as *Listeria*, is the predominance of low freezing-point branched-chain FAs in the cell membrane, which permits the maintenance of membrane function at low temperatures (Mastronicolis et al. 2005). We have also found that the cultivation at this low temperature resulted in a decrease of the total amount of produced free FAs, especially those of *iso*- and *anteiso*- chain (Fig. S5a, b in the Supplementary Material). Thus, the observed change in free FAs profile accompanying the change in temperature could be explained by the fact that at lower temperatures the incorporation of branched-chain FAs into the phospholipids of cell membrane was possibly intensified. Considering that FA synthesis in bacteria is mostly dedicated to membrane maintenance and having in mind that membrane is the first in contact with environmental stressors, it can be concluded that the remarkable tolerance of *Streptomyces* sp. NP10 strain to osmotic, cold and heat, and pH stress compared with other streptomycetes may be due to the unusual ability to biosynthesize FAs.

We have examined FA accumulation/excretion of *Streptomyces* sp. NP10 in regards to other possible ecophysiological roles such as a defense mechanism, as well as bacterial cell signaling. While FA extracts showed moderate antimicrobial properties, it significantly reduced pyocyanin production in *P. aeruginosa* PAO1 (Fig. 4a). *P. aeruginosa* regulates pyocyanin production using an intercellular communication mechanism called quorum sensing, which is a chemical communication process that bacteria use to regulate collective behaviors and in *P. aeruginosa* it is linked to pathogenicity and biofilm formation (Morkunas et al. 2012; O'Loughlin et al. 2013). Not very much is known about *Streptomyces–Pseudomonas* interspecies communication; however, it is well established that 10:1ω8c can cause dispersion of *P. aeruginosa* PAO1, *Candida* and some other microbial biofilms (Davies and Marques 2009).

As previously mentioned, free FAs have been previously reported as minor components of neutral lipids of cell membranes of few bacterial species like *Bacillus* spp. (Clejan et al. 1986), *Aquaspirillum magnetotacticum* (Gorby et al. 1988), *Streptomyces hygroscopicus* (Hoischen et al. 1997) and *Streptomyces avermitilis* (Metz et al. 1988). However, these data are very scarce except for the strain *S. avermitilis*, where FA chain length (C_{15}–C_{17}) was determined and the strain *Streptomyces* sp. A251, when percentage (up to 8 %) and identity of two fatty acids in the neutral lipid fraction were determined (Zheng et al. 2010). Very recently, an oleaginous bacterium belonging to the family *Erysipelotrichaceae* was described that produces saturated long-chain free FAs (14:0, 16:0, 18:0, and 20:0) and accumulates them as intracellular droplets in its cytoplasm. It is supposed that FAs accumulation in this strain is a result of an imbalance between excess membrane FA biosynthesis due to homeoviscous adaptation to environmental stress and limited β-oxidation activity due to anaerobic growth involving lactic acid fermentation (Katayama et al. 2014).

Therefore, it seems that even the branched FAs that have been ordinarily found as a part of the membrane phospholipids, like *i*-14:0, *a*-15:0, *i*-16:0, and etc., were detected in this study for the first time in free form in any bacteria species. Moreover, so far, ethyl esters of these common bacterial FAs, with exception of 16:0 and 18:0, were not described as metabolites produced by any microorganism. In particular, ethyl esters of *i*-12:0 and *i*-14:0 represent new natural products as they have not been found up to now in samples of natural origin, those of *i*-15:0, *a*-15:0, and *i*-16:0 were previously identified just as components of odorous secretions of the Tasmanian short-beaked echidna (Harris et al. 2012), while the derivative of *i*-10:0 was only detected in volatiles of fresh Thai green chili (Srisajjalertwaja et al. 2012).

Among the overall 59 identified free FAs (Table 1 and Table S3 in the Supplementary Material), two of them, *i*-17:0cy9-10 and *a*-18:0cy9-10, represent new natural products and the first ever found branched cyclopropane FAs.

Furthermore, branched chain monoenoic FAs, *i*-16:1ω6c, *a*-17:1ω7c, *i*-18:1ω8c, and *a*-19:1ω9c, and medium chain monoenoic FA, 13:1ω9c, have rather limited occurrence in nature (Table 2). Acid *i*-16:1ω6c was the only so far fully structurally characterized free FAs from any *Streptomyces* species. This FA and *a*-17:1ω7c were found only on few previous occasions as a part of cellular lipids of bacterial species like sulfate-reducing bacteria belonging to the genus *Desulfosarcina*, as well as *Streptomyces griseus* and/or *Legionella micdadei* (Table 2). The *i*-18:1ω8c and 13:1ω9c have not been so far identified, neither in free of bound form, in any microorganism, while there is just one previous report on the occurrence of *a*-19:1ω9c as a minor component of *Hesperopeuce mertensiana* seed triacylglicerols (Table 2). The 11:1ω1, although detected in other organisms were herein found for the first time to be produced by bacteria, while some of the identified FAs, like *i*-22:0, 3-OH-8:0, 3-OH-10:0, and 10-oxo-18:0, represented new metabolites for genus *Streptomyces*.

In recent years there is a growing interest toward the optimization of production of low-cost biofuels (FAs, alcohols, olefins, hydrocarbons, etc.) by engineered microorganisms due to the continual increase in the world's energy demands and the scarcity of fossil fuel supplies. Since microorganisms are essentially capable of synthesizing FAs, the FA production from microbial biomass is a promising and attractive alternative for traditional chemical synthesis routes (Steen et al. 2010). Although constant progress has been made in metabolic engineering strategies that are being used to improve strains performances the lipids of interest are usually accumulated within the microbial cells. Thus, it is considered that the use of microorganism that could secrete lipids into the culture media would be an effective way of simplifying the downstream processing and reducing production costs (Meng et al. 2013). Considering this, it seems that observed secretion of free FAs of this new *Streptomyces* strain into environment is quite unique and possible very applicable feature in the research field of biofuel production.

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Conflict of interest The authors declare that they have no conflicts of interest.

References

Aerts HAJ, Jacobs PA (2004) Epoxide yield determination of oils and fatty acid methyl esters using ^{1}H NMR. J Am Oil Chem Soc 81: 841–846. doi:10.1007/s11746-004-0989-1

Agar G, Adiguzel A, Baris O, Gulluce M, Sahin F (2008) Phenotypic and genetic variation of some *Salvia* species grown in eastern Anatolia region of Turkey. Asian J Chem 20:3935–3944

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402. doi:10.1093/nar/25.17.3389

Arabolaza A, Rodriguez E, Altabe S, Alvarez H, Gramajo H (2008) Multiple pathways for triacylglycerol biosynthesis in *Streptomyces coelicolor*. Appl Environ Microbiol 74:2573–2582. doi:10.1128/AEM. 02638-07

Arabolaza A, D'Angelo M, Comba S, Gramajo H (2010) FasR, a novel class of transcriptional regulator, governs the activation of fatty acid biosynthesis genes in *Streptomyces coelicolor*. Mol Microbiol 78: 47–63. doi:10.1111/j.1365-2958.2010.07274.x

Bengsch E, Perly B, Deleuze C, Valero A (1986) A general rule for the assignment of the carbon-13 NMR peaks in fatty acid chains. J Magn Reson 68:1–13. doi:10.1016/0022-2364(86)90311-2

Bernard M (2014) Molecular cloning and functional characterization of genes involved in the biosynthesis of polyunsaturated fatty acids in oat (*Avena sativa* L.). Master of Science Dissertation, University of Saskatchewan, Canada

Bhave SV, Shanbhag PV, Sonawane SK, Parab RR, Mahajan GB (2013) Isolation and characterization of halotolerant *Streptomyces radiopugnans* from Antarctica soil. Lett Appl Microbiol 56:348–355. doi:10.1111/lam.12054

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

Book AJ, Lewin GR, McDonald BR, Takasuka TE, Doering DT, Adams AS, Blodgett JA, Clardy J, Raffa KF, Fox BG, Currie CR (2014) Cellulolytic *Streptomyces* strains associated with herbivorous insects share a phylogenetically linked capacity to degrade lignocellulose. Appl Environ Microbiol 80:4692–4701. doi:10.1128/AEM. 01133-14

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

Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc Natl Acad Sci U S A 100 (Suppl 2): 14555–14561. doi:10.1073/pnas.1934677100

Chan DI, Vogel HJ (2010) Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochem J 430:1–19. doi:10.1042/BJ20100462

Chen W, Qin Z (2011) Development of a gene cloning system in a fast-growing and moderately thermophilic *Streptomyces* species and heterologous expression of *Streptomyces* antibiotic biosynthetic gene clusters. BMC Microbiol 11:243–252. doi:10.1186/1471-2180-11-243

Christie WW, Brechany EY, Marekov IN, Stefanov KL, Andreev SN (1994) The fatty acids of the sponge *Hymeniacidon sanguinea* from the Black Sea. Comp Biochem Physiol B 109:245–252. doi:10.1016/0305-0491(94)90008-6

Clejan S, Krulwich TA, Mondrus KR, Seto-Young D (1986) Membrane lipid composition of obligately and facultatively alkalophilic strains of *Bacillus* spp. J Bacteriol 168:334–340

Cole J, Wang Q, Cardenas E, Fish J, Chai B, Farris R, Kulam-Syed-Mohideen A, McGarrell D, Marsh T, Garrity G, Tiedje J (2009) The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:141–145. doi:10.1093/nar/gkn879

Davies DG, Marques CN (2009) A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol 191:1393–1403. doi:10.1128/JB.01214-08

Dembitsky VM, Rezanka T, Kashin AG (1993) Comparative study of the endemic freshwater fauna of Lake Baikal—II. Unusual lipid composition of two sponge species *Baicalospongia bacillifera* and *Baicalospongia intermedia* (family *Lubomirskiidae*, class *Demospongiae*). Comp Biochem Physiol B 106:825–831. doi:10.1016/0305-0491(93)90037-6

Dembitsky VM, Rezanka T, Kashin AG (1994) Comparative study of the endemic freshwater fauna of Lake Baikal—VI. Unusual fatty acid and lipid composition of the endemic sponge *Lubomirskia baicalensis* and its amphipod crustacean parasite *Brandtia* (*Spinacanthus*) *parasitica*. Comp Biochem Physiol B 109:415–426. doi:10.1016/0305-0491(94)90024-8

Desbois AP, Smith VJ (2010) Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol 85:1629–1642. doi:10.1007/s00253-009-2355-3

Destaillats F, Wolff RL, Angers P (2002) Saturated and unsaturated *anteiso*-C_{19} acids in the seed lipids from *Hesperopeuce mertensiana* (*Pinaceae*). Lipids 37:325–328. doi:10.1007/s11745-002-0898-y

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

Djokic L, Narancic T, Nikodinovic-Runic J, Savic M, Vasiljevic B (2011) Isolation and characterization of four novel Gram-positive bacteria associated with the rhizosphere of two endemorelict plants capable of degrading a broad range of aromatic substrates. Appl Microbiol Biotechnol 91:1227–1238. doi:10.1007/s00253-011-3426-9

Drake DR, Brogden KA, Dawson DV, Wertz PW (2008) Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. J Lipid Res 49:4–11. doi:10.1194/jlr. R700016-JLR200

Duque C, Cepeda N, Martínez A (1993) The steryl ester and phospholipid fatty acids of the sponge *Agelas conifera* from the Colombian Caribbean. Lipids 28:767–769. doi:10.1007/BF02536002

Florova G, Kazanina G, Reynolds KA (2002) Enzymes involved in fatty acid and polyketide biosynthesis in *Streptomyces glaucescens*: role of FabH and FabD and their acyl carrier protein specificity. Biochemistry 41:10462–10471. doi:10.1021/bi0258804

Gago G, Diacovich L, Arabolaza A, Tsai S-C, Gramajo H (2011) Fatty acid biosynthesis in actinomycetes. FEMS Microbiol Rev 35:475–497. doi:10.1111/j.1574-6976.2010.00259.x

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

Gorby YA, Beveridge TJ, Blakemore RP (1988) Characterization of the bacterial magnetosome membrane. J Bacteriol 170:834–841

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

Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 119:203–210. doi:10.1016/0022-1759(89)90397-9

Harris RL, Davies NW, Nicol SC (2012) Chemical composition of odorous secretions in the Tasmanian short-beaked echidna (*Tachyglossus aculeatus setosus*). Chem Senses 37:819–836. doi:10.1093/chemse/bjs066

Hertweck C (2009) The biosynthetic logic of polyketide diversity. Angew Chem Int Ed 48:4688–4716. doi:10.1002/anie.200806121

Hibbing ME, Fuqua C, Parsek MR, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8:15–25. doi:10.1038/nrmicro2259

Hoischen C, Gura K, Luge C, Gumpert J (1997) Lipid and fatty acid composition of cytoplasmic membranes from *Streptomyces hygroscopicus* and its stable protoplast-type L form. J Bacteriol 179:3430–3436

Hopwood DA (1988) The Leeuwenhoek lecture, 1987. Towards an understanding of gene switching in *Streptomyces*, the basis of sporulation and antibiotic production. Proc R Soc Lond B Biol 235:121–138. doi:10.1098/rspb.1988.0067

Kaneda T (1991) *Iso*- and *anteiso*-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol Rev 55:288–302

Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A (2008) A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr Microbiol 57:503–507. doi:10.1007/s00284-008-9276-8

Katayama T, Kanno M, Morita N, Hori T, Narihiro T, Mitani Y, Kamagata Y (2014) An oleaginous bacterium that intrinsically accumulates long-chain free fatty acids in its cytoplasm. Appl Environ Microbiol 80:1126–1131. doi:10.1128/AEM. 03056-13

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

Knief C, Altendorf K, Lipski A (2003) Linking autotrophic activity in environmental samples with specific bacterial taxa by detection of ^{13}C-labelled fatty acids. Environ Microbiol 5:1155–1167. doi:10.1046/j.1462-2920.2003.00510.x

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

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow MM (eds) Nucleic acid techniques in bacterial systematic. Wiley, Chichester, pp 115–175

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. doi:10.1093/bioinformatics/btm404

Lee JH, Vanguru M, Moore DA, Kannan G, Terrill TH, Kouakou B (2012) Flavor compounds and quality parameters of chevon as influenced by *Sericea Lespedeza* hay. J Agric Food Chem 60:3934–3939. doi:10.1021/jf2050125

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

Mastronicolis SK, Arvanitis N, Karaliota A, Litos C, Stavroulakis G, Moustaka H, Tsakirakisa A, Heropoulos G (2005) Cold dependence of fatty acid profile of different lipid structures of *Listeria monocytogenes*. Food Microbiol 22:213–219. doi:10.1016/j.fm.2004.08.002

Mei S, Ni H-M, Manley S, Bockus A, Kassel KM, Luyendyk JP, Copple BL, Ding WX (2011) Differential roles of unsaturated and saturated fatty acids on autophagy and apoptosis in hepatocytes. J Pharmacol Exp Ther 339:487–498. doi:10.1124/jpet.111.184341

Meng X, Shang H, Zheng Y, Zhang Z (2013) Free fatty acid secretion by an engineered strain of *Escherichia coli*. Biotechnol Lett 35:2099–2103. doi:10.1007/s10529-013-1305-4

Metz PA, Omstead DR, Kaplan L, Liesch JM, Stearns RA, VanDenheuvel WJ (1988) Characterization of a lipid-rich fraction synthesized by *Streptomyces avermitilis*. J Chromatogr 441:31–44. doi:10.1016/S0021-9673(01)84652-5

Moghaddam MF, Motoba K, Borhan B, Pinot F, Hammock BD (1996) Novel metabolic pathways for linoleic and arachidonic acid metabolism. Biochim Biophys Acta 1290:327–339. doi:10.1016/0304-4165(96)00037-2

Mohn T, Plitzko I, Hamburger M (2009) A comprehensive metabolite profiling of *Isatis tinctoria* leaf extracts. Phytochemistry 70:924–934. doi:10.1016/j.phytochem.2009.04.019

Morkunas B, Galloway WR, Wright M, Ibbeson BM, Hodgkinson JT, O'Connell KM, Bartolucci N, Della Valle M, Welch M, Spring DR (2012) Inhibition of the production of the *Pseudomonas aeruginosa*

virulence factor pyocyanin in wild-type cells by quorum sensing autoinducer-mimics. Org Biomol Chem 10:8452–8464. doi:10.1039/C2OB26501J

Moss CW, Lambert-Fair MA (1990) Reevaluation of the cellular fatty acid composition of *Legionella micdadei* Bari 2/158. J Clin Microbiol 28:389–390

Nichols PD, Shaw PM, Johns RB (1985) Determination of the double bond position and geometry in monoenoic fatty acids from complex microbial and environmental samples by capillary GC-MS of their Diels-Alder adducts. J Microbiol Meth 3:311–319. doi:10.1016/0167-7012(85)90013-2

Nichols PD, Guckert JB, White DC (1986) Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. J Microbiol Meth 5:49–55. doi:10.1016/0167-7012(86)90023-0

Nikolova N, Rezanka T, Nikolova-Damyanova B (2000) Fatty acid profiles of main lipid classes in adult *Chrysomela vigintipunctata* (Scopoli) (*Coleopterai*: *Chrysomelidae*). Z Naturforsch C 55:661–666

O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL (2013) A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. Proc Natl Acad Sci U S A 110:17981–17986. doi:10.1073/pnas.1316981110

Pelz O, Chatzinotas A, Zarda-Hess A, Abraham W-R, Zeyer J (2001) Tracing toluene-assimilating sulfate-reducing bacteria using ^{13}C-incorporation in fatty acids and whole-cell hybridization. FEMS Microbiol Ecol 38:123–131. doi:10.1111/j.1574-6941.2001.tb00890.x

Poerschmann J, Koschorreck M, Górecki T (2012) Organic matter in sediments of an acidic mining lake as assessed by lipid analysis. Part I: fatty acids. Sci Total Environ 414:614–623. doi:10.1016/j.scitotenv.2011.10.015

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

Ricchi M, Odoardi MR, Carulli L, Anzivino C, Ballestri S, Pinetti A, Fantoni LI, Marra F, Bertolotti M, Banni S, Lonardo A, Carulli N, Loria P (2009) Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. J Gastroenterol Hepatol 24:830–840. doi:10.1111/j.1440-1746.2008.05733.x

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 Spectrom 20:1997–2005. doi:10.1016/j.jasms.2009.07.020

Rütters H, Sass H, Cypionka H, Rullkötter J (2001) Monoalkylether phospholipids in the sulfate-reducing bacteria *Desulfosarcina variabilis* and *Desulforhabdus amnigenus*. Arch Microbiol 176:435–442. doi:10.1007/s002030100343

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

Saddler GS, O'Donnell AG, Goodfellow M, Minnikin DE (1987) SIMCA pattern recognition in the analysis of streptomycete fatty acids. J Gen Microbiol 133:1137–1147. doi:10.1099/00221287-133-5-1137

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

Skoko N, Vujovic J, Savic M, Papic N, Vasiljevic V, Ljubijankic G (2005) Construction of *Saccharomyces cerevisiae* strain FAV20 useful in detection of immunosuppressants produced by soil actinomycete. J Microbiol Meth 61:137–140. doi:10.1016/j.mimet.2004.11.007

Srisajjalertwaja S, Apichartsrangkoon A, Chaikham P, Chakrabandhu Y, Pathomrungsiyounggul P, Leksawasdi N, Supraditareporn W, Hirun S (2012) Color, capsaicin and volatile components of baked Thai green chili (*Capsicum annuum* Linn. var. *Jak Ka Pat*). J Agric Sci 4:75–84. doi:10.5539/jas.v4n12p75

Steen EJ, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, del Cardayre SB, Keasling JD (2010) Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 463:559–562. doi:10.1038/nature08721

Suthindhiran K, Kannabiran K (2009) Hemolytic activity of *Streptomyces* VITSDK1 spp. isolated from marine sediments in Southern India. J Mycol Med 19:77–86. doi:10.1016/j.mycmed.2009.01.001

Suutari M, Laakso S (1993) Signature GLC-MS ions in identification of Δ^5- and Δ^9-unsaturated *iso*- and *anteiso*-branched fatty acids. J Microbiol Meth 17:39–48. doi:10.1016/0167-7012(93)90077-U

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst197

Taylor J, Parkes J (1983) The cellular fatty acids of the sulphate-reducing bacteria, *Desulfobacter* sp., *Desulfobulbus* sp. and *Desulfovibvio desulfuvicans*. J Gen Microbiol 129:3303–3309. doi:10.1099/00221287-129-11-3303

Thumar JT, Dhulia K, Singh SP (2010) Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. World J Microbiol Biotechnol 26:2081–2087. doi:10.1007/s11274-010-0394-7

Tsydendambaev WD, Christie WW, Brechany EY, Vereshchagin AG (2004) Identification of unusual fatty acids of four alpine plant species from the Pamirs. Phytochemistry 65:2695–2703. doi:10.1016/j.phytochem.2004.08.021

van Wezel GP, McDowall KJ (2011) The regulation of the secondary metabolism of *Streptomyces*: new links and experimental advances. Nat Prod Rep 28:1311–1333. doi:10.1039/C1NP00003A

Vandamme P, Pot B, Gillis M, de Vos P, Kersters K, Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60:407–438

Veljković VB, Lakićević SH, Stamenković OS, Todorović ZB, Lazić ML (2006) Biodiesel production from tobacco (*Nicotiana tabacum* L.) seed oil with a high content of free fatty acids. Fuel 85:2671–2675. doi:10.1016/j.fuel.2006.04.015

Vyssotski M, Ryan J, Lagutin K, Wong H, Morgan X, Stott M (2012) A novel fatty acid, 12,17-dimethyloctadecanoic acid, from the extremophile *Thermogemmatispora* sp. (strain T81). Lipids 47:601–611. doi:10.1007/s11745-012-3668-z

Zgoda JR, Porter JR (2001) A convenient microdilution method for screening natural products against bacteria and fungi. Pharm Biol 39:221–225. doi:10.1076/phbi.39.3.221.5934

Zhang Y-M, Rock CO (2008) Membrane lipid homeostasis in bacteria. Nat Rev Microbiol 6:222–232. doi:10.1038/nrmicro1839

Zheng CJ, Sohn MJ, Chi SW, Kim WG (2010) Methyl-branched fatty acids, inhibitors of enoyl-ACP reductase with antibacterial activity from *Streptomyces* sp. A251. J Microbiol Biotechnol 20:875–880. doi:10.4014/jmb.1001.01004

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