Article

Applied Spectroscopy

Applied Spectroscopy
2017, Vol. 71(10) 2344–2352

DOI: 10.1177/0003702817709459
journals.sagepub.com/home/asp
SAGE

Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) for Rapid Determination of Microbial Cell Lipid Content: Correlation with Gas Chromatography-Mass Spectrometry (GC-MS)

Aaron Millan-Oropeza[1], Rolando Rebois[2], Michelle David[1], Fathi Moussa[3], Alexandre Dazzi[2], Jean Bleton[3], Marie-Joelle Virolle[1,*], and Ariane Deniset-Besseau[2,*]

Abstract

There is a growing interest worldwide for the production of renewable oil without mobilizing agriculture lands; fast and reliable methods are needed to identify highly oleaginous microorganisms of potential industrial interest. The aim of this study was to demonstrate the relevance of attenuated total reflection (ATR) spectroscopy to achieve this goal. To do so, the total lipid content of lyophilized samples of five *Streptomyces* strains with varying lipid content was assessed with two classical quantitative but time-consuming methods, gas chromatography–mass spectrometry (GC-MS) and ATR Fourier transform infrared (ATR FT-IR) spectroscopy in transmission mode with KBr pellets and the fast ATR method, often questioned for its lack of reliability. A linear correlation between these three methods was demonstrated allowing the establishment of equations to convert ATR values expressed as CO/amide I ratio, into micrograms of lipid per milligram of biomass. The ATR method proved to be as reliable and quantitative as the classical GC-MS and FT-IR in transmission mode methods but faster and more reproducible than the latter since it involves far less manipulation for sample preparation than the two others. Attenuated total reflection could be regarded as an efficient fast screening method to identify natural or genetically modified oleaginous microorganisms by the scientific community working in the field of bio-lipids.

Keywords

Biofuel, gas chromatography–mass spectrometry, GC-MS, attenuated total reflection Fourier transform, ATR FT-IR, infrared spectroscopy, lipid quantification, *Streptomyces*, triacylglycerol

Date received: 7 February 2017; accepted: 18 April 2017

Introduction

In recent decades, the ability of various microorganisms to accumulate oil has been actively assessed, as they could constitute an alternative to fossil petroleum as well as to biodiesel originating from seeds of oleaginous plants. The latter is being criticized since it mobilizes arable land that could be used for human or animal feeding. Thereby, as an alternative, large-scale cultivation of oleaginous microbes could be envisaged to face the need of renewable biodiesel. Nowadays, the most extensively explored microorganisms

[1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Gif-sur-Yvette cedex, France
[2]Laboratoire de Chimie Physique (LCP), CNRS, Université Paris-Sud, Orsay cedex, France
[3]Lip(Sys)[2], LETIAM[§], Univ. Paris-Sud, Université Paris-Saclay. IUT d'Orsay, Plateau de Moulon, F-91400, Orsay, France
[*]Equal contributors.
[§]Formerly included in EA4041 Groupe de Chimie Analytique de Paris-Sud.

Corresponding author:
Aaron Millan-Oropeza, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France.
Email: aaron.millan-oropeza@i2bc.paris-saclay.fr

are yeasts,[1] filamentous fungi and microalgae,[2] as well as, to a lesser extent, Actinomycetes such as *Rhodococcus*[3,4] and *Streptomyces*.[5] A systematic research of the best triacylglycerol (TAG) accumulating microorganisms is taking place worldwide. In order to accelerate this process, it is essential to develop medium or high-throughput methods to quantify lipids content in microorganisms.

Historically, lipid quantification was first carried out by gravimetric methods following solvent extraction.[6] These methods require large amounts of biomass, are time-consuming, and are prone to human error since they involve many manipulations. Other approaches involve neutral lipid staining techniques and fluorescence microscopy.[7,8] However, these methods turned out to be poorly quantitative. The appropriate methods should be as reliable but more user-friendly and faster than the classic gas chromatography coupled to mass spectrometry (GC-MS) method extensively used by the biologists working in the field of bio-lipids or its alternative widely used by the chemists, the Fourier transform infrared (FT-IR) spectroscopy used in transmission configuration.

With the FT-IR in transmission mode, the band intensities of the absorption spectrum and the oscillation strength of the bonds can be used to quantify the amounts of lipids within dehydrated samples. However, depending on the size of the analyzed objects, some scattering effects may appear leading to errors in the determination of band intensity, band width, and shifted maxima,[9] and to address these issues KBr pellets can be made. If they are well ground, the KBr pellets should provide a thin and homogenous layer of the samples. Consequently, the main drawback of the two methods mentioned above is the laborious and time-consuming sample preparation protocol. To avoid these lengthy sample preparations, we propose assessing lipid content directly on dehydrated microbial samples (without KBr) also with FT-IR spectroscopy but using attenuated total reflection (ATR) rather than transmission mode.

Attenuated total reflection spectroscopy provides a spectrum consisting of absorption bands in the mid-infrared (IR) range (400–4000 cm^{-1}), characteristic of chemical groups present in dehydrated microbial cells. However, whereas the ATR method proved to be quantitative to determine the fatty acids content in vegetable oils using multivariate partial least square (PLS) calibration models,[10–12] it is suspected to be semi-quantitative and poorly reliable for the analysis of lipids within microorganisms that contain proteins and other carbohydrates besides fatty acids.[13]

In order to determine whether these criticisms were justified or not, we assessed the total esterified fatty acid content of the same lyophilized bacterial samples with varying lipid content using ATR, FT-IR in transmission mode using KBr pellets as well as the classical GC-MS. Fourier transform infrared in transmission mode using KBr pellets is known to be as quantitative as GC-MS,[14] and both techniques provide the amount of lipid per unit of biomass (usually $\mu g\ mg^{-1}$), whereas the ATR method provides measurements as CO/amide I ratio. Therefore, a calibration curve between ATR and the other two methods had to be established to fulfill biologists' needs.

To do so, the content of total esterified fatty acids (TEFA) present in the same bacterial samples was determined with the three methods. The bacteria chosen to carry out these studies belong to *Streptomyces* genus. *Streptomyces* are Gram-positive soil-born filamentous bacteria well-known for their ability to produce antibiotics and other bioactive secondary metabolites useful for human health and agriculture.[15,16] However, they are less well-known for their ability to accumulate large reserve of storage lipids of the TAG family but some species are indeed oleaginous.[17] Considering that cultivation processes of *Streptomyces* have been mastered for decades by the pharmaceutical industry in large fermenters, the industrial production of lipids by these bacteria could be envisaged.[18–20] The five *Streptomyces* species mentioned in this study were chosen on the basis of a previous screen achieved with the GC-MS method that determined that their TEFA content were in the range of 30–150 $\mu g\ mg^{-1}$ (fivefold range). The two main goals of this study were: first, to evaluate the reliability of the ATR method to quantify the esterified fatty acid content of *Streptomyces* samples in comparison with that of the two other quantitative methods mentioned above; and second, to establish calibration curves and corresponding equations between the ATR spectroscopy measurements and those achieved with the two other methods. Those curves will be used for the conversion of the ATR values, expressed as CO/amide I ratio, into μg of lipid per mg of biomass.

Materials and Methods

Strains

The five *Streptomyces* strains analyzed were: *S. coelicolor* M1144, *S. coelicolor* M1142, *S. ambofaciens* OS Maroc J5, *S. pristinaespiralis* ATCC 25486, and *S. acrimycini* JI2236. The strains *S. coelicolor* M1144 and *S. coelicolor* M1142 are derivative mutants of the model strain *S. coelicolor* M145.[21] In *S. coelicolor* M1142, the biosynthetic pathways directing the synthesis of actinorhodin (ACT), undecylprodigiosin (RED), and a cryptic type I polyketide (CPK) were deleted whereas in *S. coelicolor* M1144 only the ACT and RED clusters were deleted. *S. ambofaciens* is known for the production of the macrolide spiramycin and the pyrrolamide congocidine.[22] *S. pristinaespiralis* is an industrial strain known for synthesizing pristinamycin[23] and *S. acrimycini* is known for the production of candicidin.[24]

Cell Culture and Experimental Design

Streptomyces strains were cultured on the surface of cellophane disks laid down on solid R2YE medium[25] with glycerol 0.2 mol L^{-1} or glucose 0.1 mol L^{-1} as major carbon sources and limited in phosphate (no P added) to favor TAG accumulation. 10^6 spores were plated on the surface of plates that were incubated at 28 °C for 72 h. *S. coelicolor* M1144, *S. coelicolor* M1142, *S. pristinaespiralis*, and *S. acrimycini* were cultivated on R2YE medium with glycerol 0.2 mol L^{-1} while *S. ambofaciens* and *S. coelicolor* M1144 were grown on R2YE medium with glucose 0.1 mol L^{-1}. These strains and conditions were chosen because of a previous screen achieved with the GC-MS method that determined that their TEFA content was in the range of approximately 30–150 μg mg^{-1} (fivefold range). Each condition was performed in three biological replicates.

Fourier Transform Infrared Measurements

Samples of *Streptomyces* mycelium obtained from the cultures described above were lyophilized and hermetically stored in polypropylene tubes at room temperature (20 °C) in order to minimize the hydration of the samples. Indeed, water is characterized by important absorption bands centered at 3400 cm^{-1} and at 1645 cm^{-1} that could lead to misinterpretation of the spectra. The lyophilized mycelia samples were subjected to IR spectroscopy using a Bruker Vertex 70 FT-IR spectrometer with a liquid-nitrogen-cooled MCT detector. A reference without biological sample was performed before each sample analysis. The reference and the sample analysis were carried out using 100 averaged scans conducted from 4000 cm^{-1} to 400 cm^{-1} with a spectral resolution of 4 cm^{-1}. The absorbance is given with a precision of less than 1%.

The recorded spectra showed several important absorption bands (Fig. 1). Among them, the absorption band at 1740 cm^{-1} is characteristic of the stretching of C=O esters function from esterified fatty acids. The absorption band at 1650 cm^{-1} is characteristic of the stretching of C=O and C–N bonds from proteins (amide I), and the absorption band at 1550 cm^{-1} is characteristic of the stretching of C–N bonds and the bending of N–H bonds (amide II), respectively (Fig. 1a). The absorption bands at 2955 cm^{-1}, 2873 cm^{-1} and 2923 cm^{-1}, 2853 cm^{-1} are characteristic of the antisymmetric and symmetric stretching modes of the C–H bond from the $–CH_3$ and $–CH_2$ bonds, respectively (from the carbon chain of fatty acids for example; Fig. 1b). To evaluate the lipid content of the strains, the C–H absorption bands at 2953–2853 cm^{-1} or the absorption band at 1740 cm^{-1} can be used.

Infrared Transmission Measurements of KBr pellets

For measurements involving KBr pellets, 1 mg of the lyophilized biomass was mixed with 100 mg of ground KBr (Sigma-Aldrich, ref. 221864). After grinding the mixture, the powder was pressed in a die at 10 tons for 2 min. The resulting 12.5 mm pellets were stored in a stove at 50 °C prior to analysis (to limit water absorption). The amount of esterified fatty acids is proportional to the intensity of the absorption band at 1740 cm^{-1}, taking into account that the absorption band of amide I at 1650 cm^{-1} has a contribution to the intensity of the absorption band at 1740 cm^{-1}. The tail of the amide I absorption band can be fitted using an exponential decay using Origin 8.0 software and then subtracted from the spectrum. Then, in order to determine the amount of esterified fatty acids, the absorbance value was reported to a calibration curve obtained from commercial tripalmitin (Sigma-Aldrich, ref. T5888) with amounts in the range of 0–1.45 mg (Fig. S1). Tripalmitin is a triglyceride derived from the palmitic acid (C16:0); we selected this compound as standard since C16-length lipids are the most abundant fatty acid species in the five *Streptomyces* strains used in this study (Table S1). The calibration curve showed a statistically significant *P* value of the linear regression ($P = 2.4$ e-6, ANOVA test) and had the following equation:

$$y = 1.66\,x \tag{1}$$

where *y* is tripalmitin concentration in mg per mg of dry biomass and *x* is the intensity of the absorption band at 1740 cm^{-1} of each spectrum after mathematical treatment of the different spectra. With this calibration curve, it is possible to estimate the μg of esterified fatty acids per mg of biomass using the absorbance at 1740 cm^{-1}.

Attenuated Total Reflection Measurements

For the ATR measurements, lyophilized samples were carefully ground and deposited on the diamond/ZnSe internal reflection element (IRE) of a Pike Miracle single-reflection ATR accessory without any further treatment. To improve the contact, a mechanical high-pressure clamp with a flat tip was used to press the solid samples onto the IRE (maximum pressure 689.18 bar). The applied pressure was the same as a ratchet-type clutch mechanism controls the maximum allowable pressure and the single-reflection sampling plate (1.8 mm round crystal surface) was fully covered. Even if all the experiments were carefully carried out, the contact between the IRE and the sample can vary slightly from one measurement to another. Due to this contact variation, the absorbance is given with an accuracy of 2% (technical variation). Still the deviation is better than the deviation observed due to biological variation in the three biological replicates (accuracy of 4%).

To evaluate the total esterified fatty acids content of the strains, the absorption band at 1740 cm^{-1} characteristic of the C=O stretching mode from the esterified fatty acids (Fig. 1) or the absorption band at 2923 cm^{-1} characteristic of the antisymmetric CH_2 stretching mode was used.

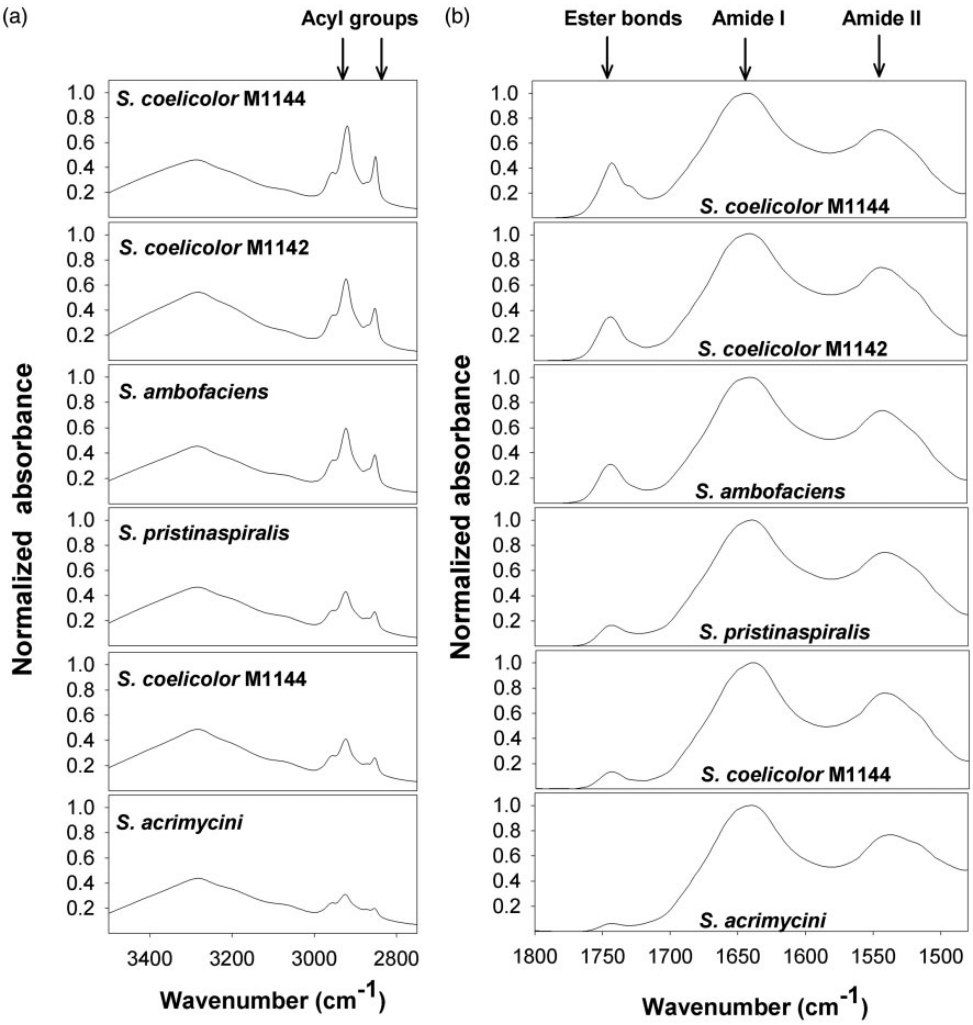

Figure 1. Recorded ATR spectra of the analyzed *Streptomyces* strains. To evaluate the total esterified fatty acids content of the different mycelial samples, the C=O absorption band at 1740 cm^{-1} (a) or the C–H absorption bands at 2953–2853 cm^{-1} can be used (b).

Subsequently, a fast and most effective data processing was applied. At first glance, a simple polynomial first order baseline correction was applied using OPUS 6.5 software and both absorption bands (at 1740 cm^{-1} and 2923 cm^{-1}) were normalized using the absorption band of amide I at 1650 cm^{-1}. An assessment of the quantity of lipids within the samples was obtained expressed as CO/amide I (if considering 1740 cm^{-1}) or CH/amide I ratio (if considering 2923 cm^{-1}).

Esterified Fatty Acids Quantification Using Gas Chromatography–Mass Spectrometry

The method used to trans-esterify the fatty acids was adapted from Lepage and Roy.[26] Samples of lyophilized *Streptomyces* mycelium (1 mg) were placed in 2 mL glass vials equipped with a screw cap. Then 180 μL of methanol, 10 μL of acetyl chloride, and 40 μL of first internal standard solution (0.3082 μg methyl tridecanoate μL^{-1}) were added to the samples. The mixture was homogenized by vortexing and incubated at 100 °C for 1 h in order to allow the acidic transesterification to take place. A total of 200 μL of hexane and 100 μL of water were added to the resulting mixture. The mixture was homogenized by vortexing and centrifuged (5 min at 2990 g). The upper hexane phase was transferred to another vial and a second extraction was performed to obtain a final fatty acid methyl esters (FAME) extract of 400 μL. The FAME extract was dried under nitrogen flux and dissolved in 400 μL of hexane to which 40 μL of a second internal standard solution (0.266 μg methyl eicosanoate μL^{-1}) was added. Aliquots of 1 μL were injected in a TRACE GC Ultra Gas Chromatograph coupled to a DSQ II quadrupole mass spectrometer (Thermo Scientific). The separation was achieved using a 20 m × 0.18 mm HP-5MS capillary column (Agilent technologies) coated with 0.18 μm of 5%-

diphenyl-95%-dimethylpolysiloxane. The carrier gas was helium at a constant pressure of 120 kPa. The injector temperature was set at 250 °C and the sample injected in splitless mode (0.5 min). The oven temperature was progressively increased from 100 °C to 310 °C at 20 °C min^{-1} then kept 2 min at 310 °C. The mass spectrometer (MS) transfer line and ion source temperatures were 325 °C and 250 °C, respectively. The MS was operated in the electron ionization (EI) positive mode (70 eV) with a scan in the range of m/z 45–650 at two scans/s. The compounds were identified by comparing the obtained mass spectra to the mass spectra of the National Institute of Standards and Technology (NIST) library.[27]

For the quantification of FAME, the peak areas were normalized using the first internal standard (C13:0) and each FAME concentration was calculated according to the following equation:

$$L_i = A_i A_{C20}^{-1} C_{C20} V_{C20} B^{-1} \quad (2)$$

where L_i is the FAME concentration in μg per mg of dry biomass, A_i is the normalized peak area of the FAME, A_{C20} is the peak area of the second internal standard (C20:0), C_{C20} represents the second internal standard concentration (0.266 μg of C20:0 μL^{-1}), V_{C20} is the added volume of second internal standard (40 μL), and B is the amount of biomass amount (mg) used for the extraction.

Total fatty acid production was obtained from the sum of all the FAME concentrations of the different FAME species present in each gas chromatogram (Figs. S2 and S3):

$$FA = \sum_{i}^{n} L_i \quad (3)$$

where FA represents the total fatty acids production in μg per mg of dry biomass, i is the FAME specie, n the total FAME species, and L_i is the FAME concentration in μg per mg of dry biomass.

Results and Discussion

Correlation Between the Quantification of Total Esterified Fatty Acid Content with Fourier Transform Infrared in Transmission and Attenuated Total Reflection Methods

Infrared spectroscopy in transmission mode is considered as a reliable and quantitative method[14] but it requires the laborious and time-consuming making of KBr pellets. In an attempt to overcome this constraint, the total fatty acids content of *Streptomyces* strains with varying levels of lipids was estimated by FT-IR spectrometry both by transmission and by ATR, which is faster and does not require any specific sample preparation besides lyophilization. The comparative analysis of the estimations provided by both techniques is shown in Fig. 2a. It represents the correlation between the μg of esterified fatty acids per mg of biomass given by the KBr pellets compared with the CO/amide I ratio obtained by ATR. Both techniques show a high correlation ($R^2 = 0.981$) resulting in the following equation:

$$y_a = 389.32\, x_a + 8.75 \quad (4)$$

where y_a represents the μg of esterified fatty acids per mg of biomass and x_a is the CO/amide I ratio from ATR. Furthermore, we observed a linear correlation ($R^2 = 0.951$, Fig. 2b) between the CO/amide I ratios of the FT-IR in transmission mode (using KBr pellets) and the CO/amide I ratios of the ATR configuration. Standard error of the mean (SEM) was calculated according to the following equation:

$$\mathrm{SEM} = \pm t_\alpha \, \mathrm{CV}\left(N^{-0.5}\right) \quad (5)$$

with $t\alpha = 1.96$ being the student coefficient as function of α-error ≤ 5% for a confidence interval of 95%, CV is the coefficient of variation and N the number of samples. These calculations resulted in SEM in the range of 0.7–8.6% and 9.1–11.4% for FT-IR in transmission and ATR, respectively. Fourier transform infrared in transmission showed less variability than ATR. However, SEM of 10%, observed for ATR, is commonly accepted in biological studies. Our results interconnected both techniques and allowed us to propose a calibration curve that can be used for quantitative and rapid measurements of total lipid content with ATR technique.

Correlation Between the Quantification of Total Esterified Fatty Acid Content with Gas Chromatography–Mass Spectrometry and Attenuated Total Reflection Methods

Gas chromatography–mass spectrometry is the method used most extensively by the biologists to assess lipid content of living organisms. The GC-MS method gives information regarding the chain length and degree of saturation/unsaturation of the fatty acids extracted from the bacteria whose summing up provides a good estimation of the total amount of esterified fatty acids. The estimations of the esterified fatty acid content obtained with the GC-MS and ATR (CO/amide I ratio) methods were compared for each strain (Fig. 3). Values obtained by ATR exhibited less SEM variation (9.1–11.4%) than those obtained with GC-MS (0.9–17.3%), probably because the latter involves more manipulations than the former. A good linear correlation was observed between CO/amide I ratios and total

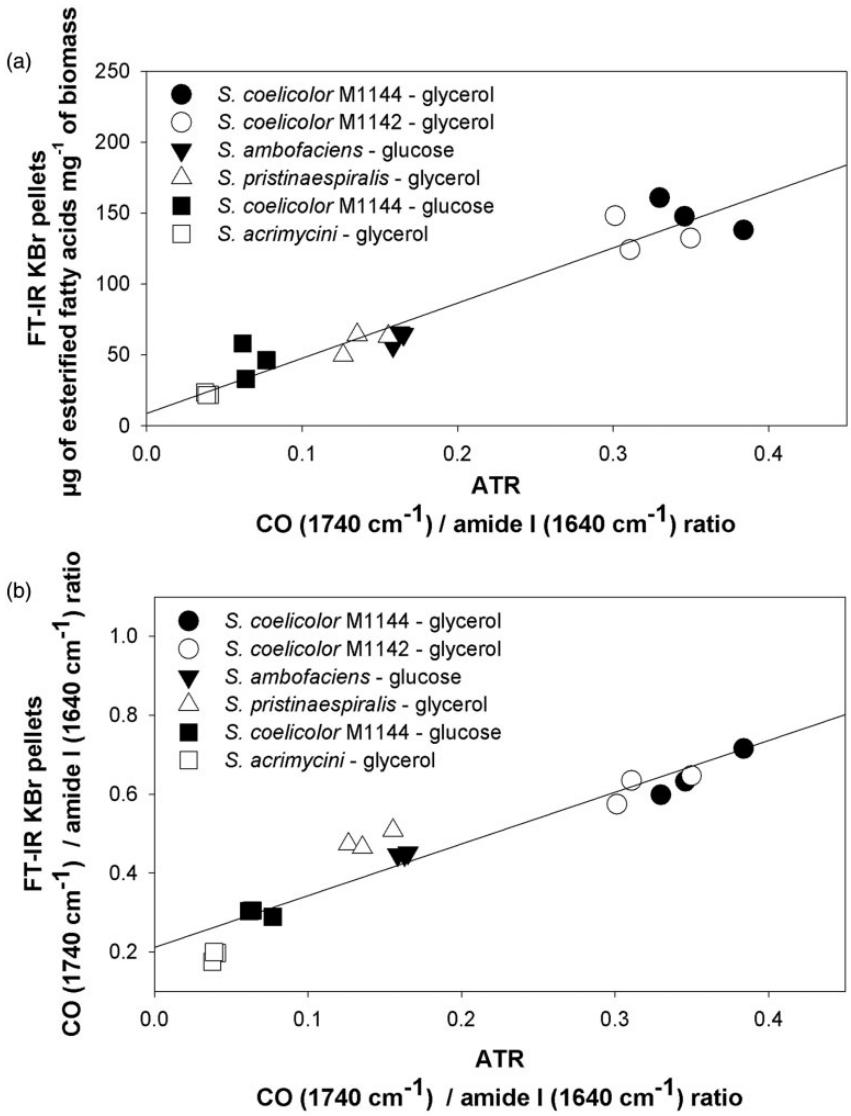

Figure 2. Correlation between CO/amide I ratios determined by ATR with the esterified fatty acids content (a) and the CO/amide I ratios (b) determined by FT-IR in transmission using KBr pellets of the analyzed *Streptomyces* strains.

FAME content as assayed by GC-MS ($R^2 = 0.992$) and expressed in the following equation:

$$y_b = 352.06\, x_b + 22.72 \quad (6)$$

where y_b represents the FAME in µg per mg of dry biomass and x_b is the CO/amide I ratio from ATR FT-IR.

Similarly, a good linear correlation was obtained between CH/amide I ratio (bands at 2923 cm^{-1} and 1650 cm^{-1}, respectively) and total FAME content assayed by GC-MS and expressed in the following equation:

$$y_c = 324.27\, x_c - 59.52 \quad (7)$$

where y_c represents the FAME in µg per mg of dry biomass and x_c is the CH/amide I ratio from ATR. The obtained correlation coefficient was $R^2 = 0.995$. The calculation of TEFA content using these two equations gave exactly the same values indicating that the other cellular components do not interfere in this assay. In another study where the lipid content of different microalgae strains was comparatively assessed using FT-IR and gravimetric methods,[28] the authors were forced to use the characteristic band of acyl groups in the range of 3000–2800 cm^{-1} to quantify the lipid content since the presence of pigments such as chlorophylls and carotenoids absorbing at 1740 cm^{-1} distorted the estimation of total lipid content using the CO/amide I ratio.

Interestingly, the GC-MS analysis revealed similar lipid profiles of the *Streptomyces* strains at quantitative (Fig. 4) and qualitative (Table S1) levels. Using this method, the ratio of lipid content between the least and most

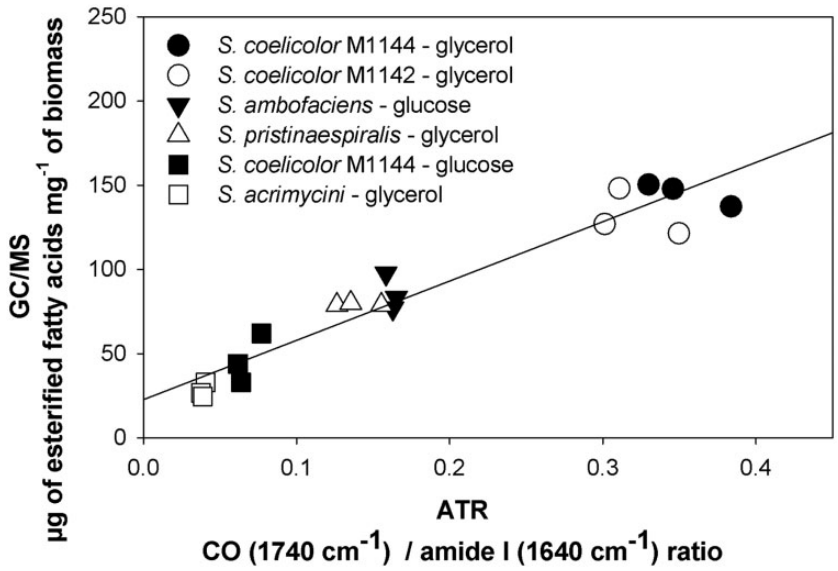

Figure 3. Correlation between the CO/amide I ratios obtained with ATR and total FAME quantification as determined by GC-MS in the analyzed *Streptomyces* samples.

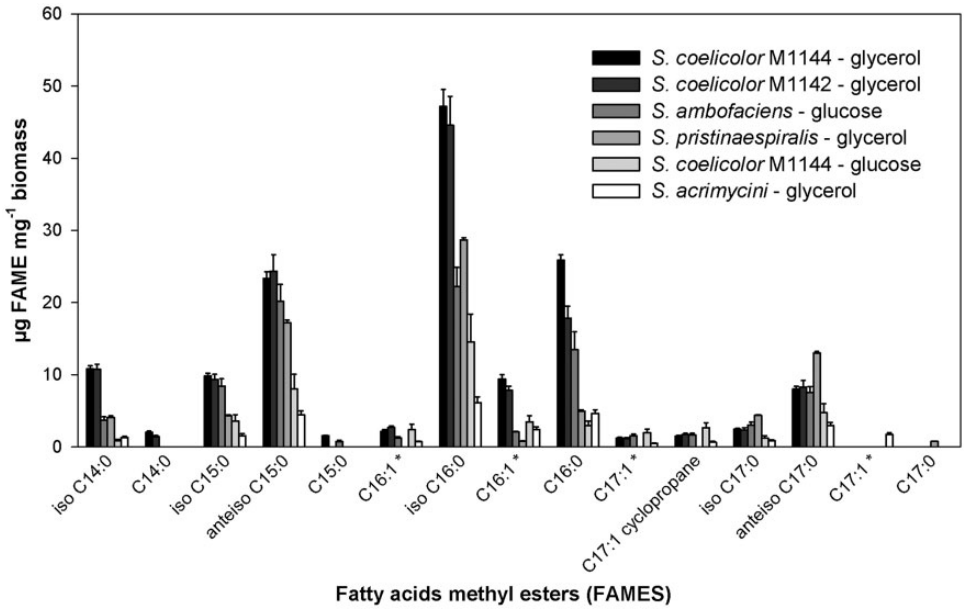

Figure 4. FAME accumulation in the analyzed *Streptomyces* strains, determined by GC-MS, showing mean and standard deviation (n = 3). *Different methyl branched unsaturated FAME for C16 and C17.

oleaginous strain was 5, in the range of 30–150 µg of esterified fatty acids per mg of dry biomass. The most abundant FAME were anteiso C15:0, iso C16:0, and C16:0. These results are consistent with other studies carried out in *S. coelicolor* and *S. ambofaciens*.[29,30] It is noteworthy that 90% of the FAME detected in the most oleaginous *Streptomyces* strains was saturated, suggesting that these bacteria could be a promising renewable oil source fulfilling the biodiesel production specifications.

Our results interconnected both techniques and allowed us to propose a calibration curve that can be used for quantitative and rapid measurements of total lipid content with ATR technique.

Conclusion

In this study we assessed the suitability and reliability of the fast ATR method to quantify TEFA content of biological

samples, in comparison with the two well recognized quantitative methods, GC-MS and FT-IR in transmission.[31] Our study demonstrated a linear correlation between GC-MS, FT-IR in transmission (using KBr pellets), and ATR methods (Figs. 2 and 3) using the representative and well resolute absorption bands of esterified fatty acids either at 1740 cm^{-1} (CO absorption band) or 2923 cm^{-1} (CH absorption band). These were used to quantify the TEFA content of *Streptomyces* strains containing approximately 30–150 μg of esterified fatty acids per mg of dry biomass.

The ATR method thus allows the direct and rapid assessment of the total amount of esterified fatty acids that include indiscriminately polar membrane lipids and neutral storage lipids (TAG). To establish the repartition of TEFA between these two pools other preparative and analytical methods are required.[32–34] The ATR method cannot provide the distribution of fatty acids chain length as do the standard methods GC-MS or GC/ flame ionization detector (FID). However, the ATR method bears many advantages in comparison with the laborious and time-consuming quantitative GC-MS and FT-IR in transmission methods: (1) it results in reliable measures of TEFA content in microorganisms as the FT-IR in transmission or GC-MS approaches if proper experimental precautions described in materials and methods are used; (2) it is highly reproducible since it involves few manipulations; and (3) it reduces substantially the processing time since signal treatment can be limited. For all these reasons, ATR is well suited for medium/high-throughput screening of oleaginous microorganisms or to follow evolution of lipid content throughout growth of microbial cells. At last, we wish to stress that the linear correlation curves obtained between the fast ATR method and the most widely accepted GC-MS or FT-IR in transmission methods are of great interest for the scientific community working in the field of bio-lipids.

Conflict of Interest

The authors report there are no conflicts of interest.

Funding

This work was supported by the Université Paris-Sud, the CNRS, and the ANR Bio-SoundIR project, grant ANR-15-CE09-0002-01 of the French National Research Agency. AMO and RR were supported by the doctoral fellowships of the National Science and Technology Council (CONACYT, Mexico) number 314457 and the Université Paris-Sud, France, respectively.

Supplemental Material

All supplemental material mentioned in the text, consisting of Figs. S1–S3 and Table S1, is available in the online version of the journal.

References

1. Q. Zhu, E.N. Jackson. "Metabolic Engineering of *Yarrowia lipolytica* for Industrial Applications". Curr. Opin. Biotechnol\. 2015. 36: 65–72.
2. C.R. Fischer, D. Klein-Marcuschamer, G. Stephanopoulos. "Selection and Optimization of Microbial Hosts for Biofuels Production". Met. Eng. 2008. 10: 295–304.
3. K. Kurosawa, A. Radek, J.K. Plassmeier, et al. "Improved Glycerol Utilization by a Triacylglycerol-producing *Rhodococcus opacus* Strain for Renewable Fuels". Biotechnol. Biofuels. 2015. 8: 1–11.
4. S.A. Shields-Menard, M. Amirsadeghi, B. Sukhbaatar, et al. "Lipid Accumulation by *Rhodococcus rhodochrous* Grown on Glucose". J. Ind. Microbiol. Biotechnol. 2015. 42: 693–639.
5. A. Arabolaza, E. Rodriguez, S. Altabe, et al. "Multiple Pathways for Triacylglycerol Biosynthesis in *Streptomyces coelicolor*". Appl. Environ. Microbiol. 2008. 74: 2573–2582.
6. E.G. Bligh, W.J. Dyer. "A Rapid Method of Total Lipid Extraction and Purification". Can. J. Biochem. Phys. 1959. 37: 911–917.
7. W. Chen, C. Zhang, L. Song, et al. "A High Throughput Nile Red Method for Quantitative Measurement of Neutral Lipids in Microalgae". J. Microbiol. Methods. 2009. 77: 41–47.
8. T. Govender, L. Ramanna, I. Rawa, et al. "BODIPY Staining, an Alternative to the Nile Red Fluorescence Method for the Evaluation of Intracellular Lipids in Microalgae". Bioresour. Technol. 2012. 114: 507–511.
9. A. Dazzi, A. Deniset-Besseau, P. Lasch. "Minimising Contributions from Scattering in Infrared Spectra by Means of an Integrating Sphere". Analyst. 2013. 138: 4191–4201.
10. S.T.H. Sherazi, S. Arain, S.A. Mahesar, et al. "Erucic Acid Evaluation in Rapeseed and Canola Oil by Fourier Transform-Infrared Spectroscopy". Eur. J. Lip. Sci. Technol. 2013. 115: 535–540.
11. M.M. Mossoba, H. Azizian, C. Tyburczy, et al. "Rapid FT-NIR Analysis of Edible Oils for Total SFA, MUFA, PUFA, and trans FA with Comparison to GC". J. Am. Oil. Chem. Soc. 2013. 90: 757–770.
12. S.T.H. Sherazi, M.Y. Talpur, S.A. Mahesar, et al. "Main Fatty Acid Classes in Vegetable Oils by SB-ATR-Fourier Transform infrared (FTIR) Spectroscopy". Talanta. 2009. 80: 600–606.
13. A.M.A. Pistorius, W.J. DeGrip, T.A. Egorova-Zachernyuk. "Monitoring of Biomass Composition from Microbiological Sources by Means of FT-IR Spectroscopy". Biotechnol. Bioeng. 2009. 103: 123–129.
14. G. Ramer, B. Lendl. "Attenuated Total Reflection Fourier Transform Infrared Spectroscopy". Encyclopedia of Analytical Chemistry. Chichester, UK: John Wiley and Sons, Ltd, 2006.
15. J. Berdy. "Bioactive Microbial Metabolites". J. Antibiot. 2005. 58: 1–26.
16. M.J. Bibb. "Regulation of Secondary Metabolism in Streptomycetes". Curr. Opin. Microbiol. 2005. 8: 208–215.
17. E.R. Olukoshi, N.M. Packter. "Importance of Stored Triacylglycerols in *Streptomyces*: Possible Carbon Source for Antibiotics". Microbiol. 1994. 140: 931–943.
18. M. Holmback, M. Lehesto, P. Koskinen, et al. "Process and Microorganisms for Production of Lipids". European patent 148056. Neste Oil Oyj, 2014.
19. F. Thevenieau, M.J. Virolle, T. Dulermo, et al. "Method for the Production of Lipids by Microorganisms and Use of Said Lipids". European patent 202778.2014.
20. F. Thevenieau, S. Sambou, M.J. Virolle, et al. "Triacylglycerol-based Lipid Composition". United States 203219. AVRIL, Universite Paris-Sud, 2014.
21. J.P. Gomez-Escribano, M.J. Bibb. "Engineering *Streptomyces coelicolor* for Heterologous Expression of Secondary Metabolite Gene Clusters". Microb. Biotechnol. 2011. 4: 207–215.
22. B. Aigle, S. Lautru, D. Spiteller, et al. "Genome Mining of *Streptomyces ambofaciens*". J. Ind. Microbiol. Biotechnol. 2014. 41: 251–263.
23. N. Bamas-Jacques, S. Lorenzon, P. Lacroix, et al. "Cluster Organization of the Genes of *Streptomyces pristinaespiralis* Involved in Pristinamycin Biosynthesis and Resistance Elucidated by Pulsed-field Gel Electrophoresis". J. Appl. Microbiol. 1999. 87: 939–948.
24. J.A. Asturias, J.F. Martín, P. Liras. "Biosynthesis and Phosphate control of Candicidin by *Streptomyces acrimycini* JI2236: Effect of Amplification of the pabAB Gene". J. Ind. Microbiol. 1994. 13: 183–189.

25. T. Kieser, M.J. Bibb, M.J. Buttner, et al. "Practical *Streptomyces* Genetics". Norwich, UK: John Innes Foundation, 2000.
26. G. Lepage, C.C. Roy. "Direct Transesterification of all Classes of Lipids in a One-Step Reaction". J. Lip. Res. 1986. 27: 114–120.
27. National Institute of Standards and Technology (NIST). "Standard Reference Database 14". http://www.nist.gov/srd/nist1a.cfm, 2014.
28. A.P. Dean, D.C. Sigee, B. Estrada, et al. "Using FTIR Spectroscopy for Rapid Determination of Lipid Accumulation in Response to Nitrogen Limitation in Freshwater Microalgae". Bioresour Technol. 2010. 101: 4499–4507.
29. Y. Li, G. Florova, K.A. Reynolds. "Alteration of the Fatty Acid Profile of *Streptomyces coelicolor* by Replacement of the Initiation Enzyme 3-Ketoacyl Acyl Carrier Protein Synthase III (FabH)". J. Bacteriol. 2005. 187: 3795–3799.
30. C. Schauner, A. Dary, A. Lebrihi, et al. "Modulation of Lipid Metabolism and Spiramycin Biosynthesis in *Streptomyces ambofaciens* Unstable Mutants". Appl. Environ. Microbiol. 1999. 65: 2730–2737.
31. H. Azizian, J.K.G. Kramer, M.M. Mossoba. "Evaluating the Transferability of FT-NIR Calibration Models for Fatty Acid Determination of Edible Fats and Oils Among Five Same-make Spectrometers Using Transmission or Transflection Modes with Different Pathlengths". J. Am. Oil Chem. Soc. 2012. 89: 2143–2154.
32. S.K. Abbott, P.L. Else, T.A. Atkins, et al. "Fatty Acid Composition of Membrane Bilayers: Importance of Diet Polyunsaturated Fat Balance". BBA-Biomembranes. 2012. 1818: 1309–1317.
33. S.M. Heinzelmann, N.J. Bale, E.C. Hopmans, et al. "Critical Assessment of Glyco-and Phospholipid Separation by Using Silica Chromatography". Appl. Environ. Microbiol. 2014. 80: 360–365.
34. R. Michael-Jubeli, J. Bleton, A. Baillet-Guffroy. "High-temperature Gas Chromatography-Mass Spectrometry for Skin Surface Lipids Profiling". J. Lip Res. 2011. 52: 143–151.