Page 262 to 266

Volume 1 • Issue 6 • 2017

Review Article

Innovative Techniques in Agriculture

ISSN: 2575-5196

Analysis of Soil Lipids for Studies of Microbial Communities

Alejandro E Ferrari* and Luis G Wall

Laboratorio de Bioquímica, Microbiología e Interacciones Biológicas en el Suelo, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, R. Sáenz Peña 352 B1876BXD Bernal, Buenos Aires, Argentina

***Corresponding Author:** Alejandro E Ferrari, Laboratorio de Bioquímica, Microbiología e Interacciones Biológicas en el Suelo, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, R. Sáenz Peña 352 B1876BXD Bernal, Buenos Aires, Argentina.

Received: October 02, 2017; **Published:** October 16, 2017

Volume 1 Issue 6 October 2017

Introduction

Lipids are a heterogenic group of natural chemical compounds, having in common low solubility in polar solvents and high in non-polar solvents. This definition includes steroids, carotenoids, terpenes and biliar acids. But also non-lipid substances like hydrocarbons. Chemical structure and biological functions of these compounds can be quite different. In a more restrictive definition, lipids are considered as fatty acids and their derivatives (amides and esters), together with compounds related through biosynthesis paths of biological function.

The most abundant lipid are the fatty acids linked by ester links to an alcohol (like glycerol), including acyl-glycerols (often called neutral lipids) and polar lipids, where one fatty acid is replaced by a sugar and a phosphate group (phospholipids).

Neutral lipids are important systems for storing energy in eukaryote organisms, while phospholipids are structural lipids in the cell membrane of every living being, constituting a double layer that allows polar molecules and ions passing through the cell.

Methodology

Lipids can be analyzed by gas or liquid chromatography.

Gas chromatography is fast, cheap and sensitive method for analysis lipids, but they must be derivatizated for increasing their volatility. It is done by alkaline saponification, where the fatty acids are converted in methyl esters fatty acids (FAMEs).

Two different approaches are used for lipid analysis:

The whole cell fatty acid (WCFA) method, sometimes called FAME or MIDI method (Sasser 1990, MIDI 2001) includes four steps: direct alkaline saponification, acid methylation, FAME extraction and extract washing.

The *phospholipid fatty* acid (PLFA) method (White., *et al*. 1979, Frostegård., *et al.* 1991) consists in a direct extraction from soil with a solvent mixture, followed by a fractionation step in a silica solid-phase extraction column (Zelles 1999). During the fractionation, three fractions are obtained: neutral lipid fatty acid (NLFA), glycolipid fatty acid (GLFA) and phospholipid fatty acid (PLFA) fractions. In general,

only the first and the last fractions (NLFA and PLFA) are analyzed. A mild alkaline methanolysis step was applied to the fractions, where the ester-bond (but not the phosphate bond) is broken.

Though the WCFA lipids can be considered as the sum of the NLFA, GLFA and PLFA, both techniques are not comparable and must be considered different methods. However, both techniques are equally skillful for comparing soils under different agricultural management.

Applications

Identification of microorganisms in pure cultures

The WCFA or MIDI method has been developed for identification of pure cultures of unknown microorganisms on an artificial media by the Sherlock Microbial Identification System (MIS or MIDI) (MIDI Inc, Newark, DE, USA) (Sasser 1990). The method compares the lipid profile with a library and with reference strains of culturable microorganisms; especially aerobes and anaerobes bacteria and yeasts.

With the name WCFA, this method was later extended to analysis of soil samples, though some controversy about which fatty acids must be used as specific *taxa* markers still persists.

It is broadly accepted that the arbuscular mycorrhizal fungi have 16:1ω5c as a signature specific marker (Grigera., *et al*. 2007, Gryndler., *et al*. 2009).

The fatty acids 18:2ω6,9c, 18:1ω9c and 18:3ω6,9,12c were also used as markers of fungi (Montecchia., *et al.* 2011).

The branched fatty acids, especially 15:0iso, 15:0anteiso, 16:0iso, 17:0iso and 17:0anteiso are considered markers of Gram-positive bacteria (Hedrick., *et al*. 2010).

Gram-negative bacteria are characterized by hydroxylated, cyclic and some MUFAs, but there exist strong differences in the selection of them. Most authors choose 17:0cy y 19:0cy as markers of Gram-negative (Pankhurst., *et al.* 2001).

For actinomycetes, the fatty acids methylated in carbon 10 are considered signature biomarkers, especially 16:010Me, 17:010Me and 18:010Me (Zelles, 1999).

The fatty acid 20:4 is a general marker of eukaryotes, especially protozoa (Ravnskov., *et al.* 2006).

Assessment of microbial biomass

Because of their high turnover as biomolecules, phospholipid fatty acids are thought to provide information about the active soil microbial community in soil samples (Tunlid & White, 1992). The PLFAs showed good correlation with total viable biomass, as these fatty acids do not have energy storage functions and they quickly degrade after cell death. Neutral lipid fatty acids, which mainly consist in energy reserve substances in living organisms, may provide information about the physiological status of soil biota.

It has been demonstrated that biomass measured as total PLFA show good correlations with other method of assessment of microbial biomass, like fumigation-extraction (Bailey., *et al*. 2002), Fumigation-incubation (Feng., *et al.* 2003), substrate induced respiration (Rinklebey Langer 2010).

There is only one limitation in this topic, when the soil is contaminated. So, toxic substances in the soil can kill the microbes and inhibit the enzymes that degrade PLFAs, delaying the degradation process (Frostegård., *et al*. 2011).

PLFAs as markers of cell stress

Bacterial cells have defined strategies for adapting to environmental stress, including the modification of their membrane fatty acids (Hedrick., *et al.* 2010, Frostegård., *et al.* 2011) in order to decrease the permeability.

In this way, many stress markers based on lipids have been proposed, like the precursor/substrate ratios; applied to different stress conditions like acidity, toxicity, anaerobiosis, high temperatures, starvation, osmotic stress y low nutrient availability (Meriles., *et al*. 2009, Romaniuk., *et al.* 2011).

According to known metabolic paths of lipid biosynthesis, the formation of trans and cyclo fatty acids from their precursors cis and MUFA, respectively, indicate environmental stress (Borjesson., *et al.* 2012), being the conversions *cisMUFA→transMUFA y cisMUFA→ ciclo* the most used (Hedrick., *et al.* 2010).

A long exposition to conditions that induce the stationary face growth has produced higher levels of cyclic PLFAs (Bossio y Scow 1998). For example, with protobacteria, the 17:0cy/16:1ω7c y 19:0cy/18:1ω7c ratios increased from about 0.05 in the log face to about 2.5 in the stationary face (Hedrick., *et al.* 2010).

Lipid as markers of agricultural management

Analysis of lipid in soil samples is a key tool for studies of microbial communities because they do not depend of microbial culture. It is supposed that only 1% of the microbial population in soil can be cultivated in the lab (Kirk., *et al*. 2004).

Either the WCFA or the PLFA methods have been used in studies of agricultural soils all over the world. The different soil agricultural managements, as levels of crop rotation, fertilization and pest control, should produce shifts in the microbial community structure and these shifts affect the lipid profiles. In this way, lipid profiles would allow the detection of specific fatty acids that can show high sensitivity to changes in agricultural management, in fields with different use history (Ferrari., *et al.* 2014).

Lipid analysis by both WCFA and PLFA methods have been use to study the effects of fertilization (Zhao., *et al*. 2015, Börjesson., *et al*. 2012), crop rotation (Meriles., *et al*. 2009, Montecchia., *et al*. 2011, Ferrari., *et al*. 2014), tillage (Ritchie., *et al*. 2000, Meriles., *et al*. 2009, Ibekwe., *et al*. 2002, van Groenigen., *et al*. 2010, Feng., *et al*. 2003), fumigants (Ibekwe., *et al*. 2001), pollution and bioremediation (Macnaughton., *et al*. 1999) and benefic-pathogen microbe interactions (Ravnskov., *et al*. 2006, Chen., *et al*. 2001, Ruess., *et al*. 2007).

References

1. Bayley VL., *et al*. "Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate induced respiration and phospholipid fatty acid analysis". *Soil Biology and Biochemistry* 34.9 (2002): 1385-1389.
2. Börjesson G., *et al*. "Soil microbial community structure affected by 53 years of nitrogen fertilization and different organic amendments". *Biology and Fertility of Soils* 48.3 (2012): 245-257.
3. Chen J., *et al.* "Fatty acid composition and dynamics of selected fungal-feeding nematodes and fungi". *Comparative Biochemistry and Physiology B* 130.2 (2001): 135-144.
4. Feng Y., *et al*. "Soil microbial communities under conventional till and no-till continuous cotton systems". *Soil Biology and Biochemistry* 35.12 (2003): 1693-1703.
5. Ferrari AE., *et al*. "Crop rotation and seasonal effects on fatty acid profiles of neutral and phospolipids extracted from no-till agricultural soils". *Soil Use & Management* 31.1 (2014): 165-175.
6. Frostegård Å., *et al.* "Microbial biomass measured as total lipid phosphate in soils of different organic content". *Journal of Microbiological Methods* 14.3 (1991): 151-163.
7. Frostegård Å., *et al.* "Use and misuse of PLFA measurements in soils". *Soil Biology and Biochemistry* 43.8 (2011): 1621-1625.
8. Grigera MS., *et al.* "Distribution of the arbuscular mycorrhizal biomarker C16:1cis11 among neutral, glycol and phospholipids extracted from soil during the reproductive growth of corn". *Soil Biology and Biochemistry* 39 (2007): 1589-1596.

9. Gryndler M., *et al*. "Growth of arbuscular mycorrhizal (AM) fungi and other microorganisms in field soil as affected by long-term mineral and organic fertilization". *Mycorrhiza* 16 (2006): 159-166.
10. Hedrick DB., *et al*. "Interpretation of fatty acid profiles of soil microorganisms. In: Manual of soil analysis–Monitoring and assessing soil bioremediation (eds R. Margesin & F. Schinner), Springer-Verlag, Berlin, Heidelberg". (2010): 251-259.
11. Ibekwe AM., *et al*. "Microbial diversity along a transect of agronomic zones". *FEMS Microbiology Ecology* 39.3 (2002): 183-191.
12. Kirk JL., *et al*. "Methods of studying soil microbial diversity". *Journal of Microbiological Methods* 58.2 (2004): 169-188.
13. MacNaughton S., *et al.* "Microbial population changes during bioremediation of an experimental oil spill". *Applied & Environmental Microbiology* 65.8 (1999): 3566-3574.
14. Meriles JM., *et al.* "Soil microbial communities under different soybean cropping systems: Characterization of microbial population dynamics, soil microbial activity, microbial biomass and fatty acid profiles". *Soil and Tillage Research* 103.2 (2009): 271-281.
15. Myron Sasser. "Identification of bacteria by gas chromatography of cellular fatty acids". *Technical note* (2001): 101.
16. Montecchia MS., *et al.* "Multivariate approach to characterizing soil microbial communities in pristine and agricultural sites in northwest Argentina". *Applied Soil Ecology* 47.3 (2011): 176-183.
17. Pankhurst CE., *et al.* "Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia". *Biology and Fertility of Soils* 33.3 (2001): 204-217.
18. Ravnskov S., *et al*. "Soil inoculation with the biocontrol agent Clonostachys rosea and the mycorrhizal fungus Glomus intraradices results in mutual inhibition, plant growth promotion and alteration of soil microbial communities". *Soil Biology and Biochemistry* 38.12 (2006): 3453-3462.
19. Rinklebe J and Langer U. "Relationship between soil microbial biomass determined by SIR and PLFA analysis in floodplain soils". *Journal of Soils and Sediments* 10.1 (2010): 4-8.
20. Ritchie NJ., *et al.* "Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities is soil". *Applied and Environmental Microbiology* 66.4 (2000): 1668-1675.
21. Romaniuk R., *et al*. Assessment of soil microbial diversity measurements as indicators of soil functioning in organic and conventional horticulture systems. *Ecological Indicators* 11.5 (2011): 1345-1353.
22. Ruess L., *et al*. "Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains". *Soil Biology and Biochemistry* 37.5 (2005): 945-953.
23. Sasser M. Identification of bacteria through fatty acid analysis. In: Methods in Phytobacteriology (eds Z. Clement, K. Rudolph, D.C. Sands). Akadémiai Kiadó, Budapest". (1990): 199-204.
24. Tunlid A and White DC. "Biochemical analysis of biomass, community structure, nutritional status and metabolic activity of microbial communities in soil". *Soil Biochemistry* 7 (1992): 229-262.
25. Van Groenigen KJ., *et al.* "Abundance, production and stabilization of microbial biomass under conventional and reduced tillage". *Soil Biology & Biochemistry* 42.1 (2010): 48-55.
26. White DC., *et al*. "Determination of the sedimentary microbial biomass by extractible lipid phosphate". *Oecologia* 40.1 (1979): 51-62.
27. Zelles L. "Fatty acid pattern of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review". *Biology and Fertility of Soils* 29.2 (1999): 111-129.