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Automated Phospholipid Fatty Acid (PLFA) Analysis using the Sherlock™ PLFA Software Package on a Shimadzu GC-2010/2030

Application Note | Soil Microbial Community Analysis

Abstract

Soil phospholipid fatty acid (PLFA) analysis can provide a real-time snapshot of the soil microbial community (soil microbiota) structure. Using a high throughput PLFA extraction method[1], coupled to an automated PLFA naming process reduces turnaround time and reagent use, while limiting potential errors that can occur with "manual" PLFA analysis approaches.

Introduction

The soil microbiota is responsible for many ecosystem functions such as plant growth regulation, nutrient cycling and carbon sequestration. Additionally, the microbiota can degrade environmental pollutants, such as PAHs and PCBs. The microbiota is highly sensitive to soil-altering processes (degradative or beneficial) and changes can guide appropriate management procedures (conservation or restoration).

Phospholipids are an essential structural component of all microbial cellular membranes. Upon microbial death, phospholipids rapidly degrade. Phospholipid content in a soil sample is therefore assumed to be from the living microbiota. Phospholipid fatty acids (PLFAs) are the main structural component of the phospholipid and can serve as useful biomarkers for different microbial groups.

PLFA analysis is a widely-used technique for estimation of the living microbial biomass and to observe broad changes in the soil microbiota composition. Multiple different Gas Chromatography (GC) and Gas Chromatography-Mass Spectroscopy (GC-MS) methods and instrument types have been used to determine PLFA profiles. However, most of these methods are performed manually, and the analysis process is laborious and potentially error-prone.

The Sherlock PLFA Analysis software automatically names the PLFAs in a sample and categorizes them by microbial origin (e.g. Actinomycetes). This automated process yields consistent and easy-to-interpret results with less chance of errors. The sensitivity of the Sherlock method ensures that all discernible PLFAs

are measured. Furthermore, the PLFA data can be visualized with the Sherlock 2-D Plot and Dendrogram analysis tools or exported to Microsoft Excel® or Access® databases for further study and ease of publication.

Experimental

This note details the PLFA analysis of a river sediment sample using the Sherlock PLFA Software Package on a Shimadzu GC-2010 Plus, following a high throughput PLFA extraction protocol[1]. A known number of moles of the internal standard (ISTD), 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (19:0 PC, Avanti Polar Lipids p/n 850367), were added at the beginning of the process to assist in the biomass calculations.

After the *PLFAD2* Method was selected from the list of available methods within the Sherlock Sample Processor, the correct method parameters were automatically loaded into the Shimadzu GC's LabSolutions software. The PLFA Calibration Standard was processed first in the batch, and the results from the calibration were used to determine the expected retention times (RT) of each PLFA in the sample.* The calibration run was followed by a hexane blank and then the sample run.

Sherlock automatically calculated each peak name and weight percent. 36 fatty acids were identified in the sample (including the ISTD) and 100.00% of the peaks were named.

Sherlock PLFA Analysis – Raw Peak Identification Report

Method: PLFAD2 File: E169023.82S
Type: Samp Bottle: 30
Created: 10/06/2016 9:01:33 AM
Sample ID: KY-RIVER-27

RT	Response	RFact	ECL	Peak Name	Wgt %
0.7304	2.311E+9	----	7.7032	SOLVENT PEAK	----
2.1444	6979	1.199	13.6124	14:0 iso	1.09
2.3028	16751	1.181	14.0000	14:0	2.59
2.5218	5023	1.160	14.4418	15:1 iso w6c	0.76
2.6081	26000	1.152	14.6160	15:0 iso	3.92
2.6553	15203	1.147	14.7113	15:0 anteiso	2.28
2.7992	5729	1.133	15.0012	15:0	0.85
3.1576	9008	1.103	15.6220	16:0 iso	1.30
3.2752	111668	1.093	15.8256	16:1 w7c	15.96
3.3267	27107	1.089	15.9148	16:1 w5c	3.86
3.3773	99814	1.084	16.0021	16:0	14.15
3.6511	19277	1.063	16.4275	16:0 10-methyl	2.68
3.7011	4577	1.060	16.5052	17:1 iso w9c	0.63
3.7785	7935	1.054	16.6254	17:0 iso	1.09
3.8409	5861	1.049	16.7223	17:0 anteiso	0.80
3.8908	4456	1.045	16.7999	17:1 w8c	0.61
3.9547	10598	1.041	16.8992	17:0 cyclo w7c	1.44
4.0211	4916	1.036	17.0022	17:0	0.67
4.4480	9125	1.006	17.6163	18:0 iso	1.20

RT	Response	RFact	ECL	Peak Name	Wgt %
4.4853	5406	1.005	17.6699	18:4 w3c	0.71
4.5266	22632	1.002	17.7293	18:2 w6c	2.97
4.5594	41106	0.999	17.7765	18:1 w9c	5.37
4.5958	100675	0.997	17.8287	18:1 w7c	13.13
4.6532	18531	0.993	17.9114	18:1 w5c	2.41
4.7158	19520	0.989	18.0015	18:0	2.53
4.7769	7215	0.985	18.0856	18:1 w7c 10-methyl	0.93
4.9996	8242	0.972	18.3922	18:0 10-methyl	1.05
5.3745	25667	0.950	18.9084	19:0 cyclo w7c	3.19
5.4428	55056	----	19.0024	19:0	0.00
5.7451	16755	0.931	19.4088	20:4 w6c	2.04
5.8018	37069	0.928	19.4850	20:5 w3c	4.50
5.9958	7496	0.918	19.7458	20:2 w6c	0.90
6.1859	4342	0.909	20.0013	20:0	0.52
7.1263	7337	0.872	21.2626	22:5 w6c	0.84
7.1866	17960	0.870	21.3440	22:6 w3c	2.04
7.6737	4198	0.856	22.0009	22:0	0.47
9.1198	4707	0.844	23.9996	24:0	0.52

Total Response: 738885 Percent Named: 100.00% Peaks Named: 36

** The expected RTs of each PLFA in the sample are calculated by assigning Equivalent Chromatographic Locales™ (ECL) to each PLFA based on previous analyses of those compounds. Known ECLs from the MIDI PLFA calibration mixture are used to convert RTs from each sample run into ECLs that can then be named based on the MIDI's PLFA Peak Naming Table.*

The Sherlock PLFA Tools software was then used to rapidly calculate the mole percent of each PLFA.

Sherlock PLFA Analysis – Peak Identification Report Converted to Mol %

Method: PLFAD2 File: E169023.WGT
Type: Samp
Created: 10/06/2016 9:04:22 AM
Sample ID: KY-RIVER-27

Nano moles	Peak Name	Mol %
2070	14:0 iso	1.28
4894	14:0	3.02
1373	15:1 iso w6c	0.85
7006	15:0 iso	4.33
4080	15:0 anteiso	2.52
1519	15:0	0.94
2204	16:0 iso	1.36
27263	16:1 w7c	16.85
6592	16:1 w5c	4.07
24011	16:0	14.84
4322	16:0 10-methyl	2.67
1030	17:1 iso w9c	0.64
1764	17:0 iso	1.09
1297	17:0 anteiso	0.80
989	17:1 w8c	0.61
2341	17:0 cyclo w7c	1.45
1074	17:0	0.66
1845	18:0 iso	1.14

Nano moles	Peak Name	Mol %
1121	18:4 w3c	0.69
4616	18:2 w6c	2.85
8310	18:1 w9c	5.14
20305	18:1 w7c	12.55
3721	18:1 w5c	2.30
3880	18:0	2.40
1373	18:1 w7c 10-methyl	0.85
1537	18:0 10-methyl	0.95
4741	19:0 cyclo w7c	2.93
10000	19:0	----
2935	20:4 w6c	1.81
6520	20:5 w3c	4.03
1280	20:2 w6c	0.79
725	20:0	0.45
1114	22:5 w6c	0.69
2735	22:6 w3c	1.69
608	22:0	0.38
623	24:0	0.39

The Sherlock PLFA Tools software was then used to scale the data to the IS (19:0), calculate the biomass (nmol/g) for each microbial type (customizable) and calculate key PLFA ratios (customizable), all within about 5 minutes after the sample run was processed through the Shimadzu GC.

Sherlock PLFA Analysis – Custom Analysis

Biomass (nmol/g) by Microbial Type

Method: PLFAD2 File: E169023.MIC
Type: Samp
Created: 10/6/2016 9:07:03 AM
Sample ID: KY-RIVER-27

PLFA Origin	Biomass (nmol/g)
Gram-Positive	30.98
Gram-Negative	59.36
AM Fungi	6.59
Fungi	4.62
Actinomycetes	7.23
Other Eukaryote	15.70
Not Assigned	37.33
Total PLFA	161.81

Key PLFA Ratios

Method: PLFAD2 File: E169023.RAT
Type: Samp
Created: 10/6/2016 9:07:23 AM
Sample ID: KY-RIVER-27

Ratio Name	Ratio
Fungi/Bacteria	0.12
Predator/Prey	0.19
Gram+/Gram-	0.50
Sat/Unsat	0.69
Mono/Poly	3.31
GNeg Stress	6.72

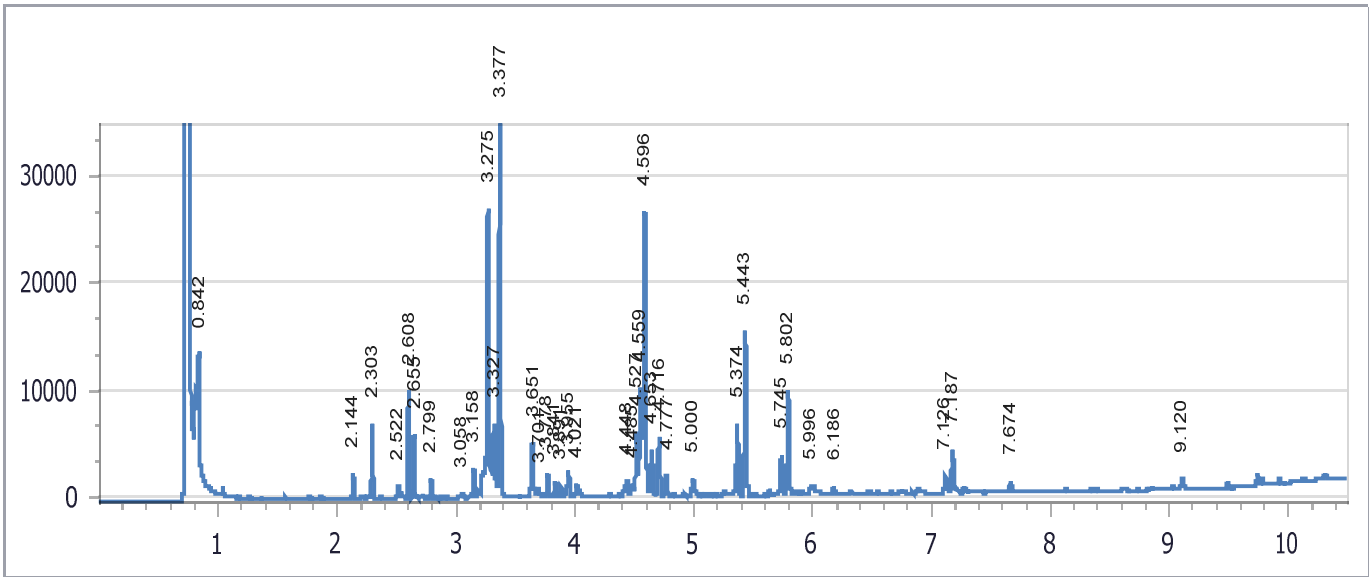

Figure 1. Representative chromatogram for sample KY-RIVER-27, with retention times labeled.

Conclusion

PLFA analysis of soil samples via the MIDI Sherlock™ PLFA Software Package with a Shimadzu GC-2010/2030 provides an automated and comprehensive method for analyzing PLFAs from the soil microbiota. Coupled to a high throughput extraction method[1], the MIDI PLFA Solution results in a rapid and standardized PLFA protocol that can be implemented by most laboratories for detailed study of the soil microbiota. User-defined variables (e.g. which fatty acids to assign to which microbial groups) allow for customization of results.

Reference

Buyer, J.S. & Sasser, M. (2012). High throughput phospholipid fatty acid analysis of soils. In *Applied Soil Ecology* **61**, 127-130.

Full Text Version
www.sciencedirect.com/science/article/pii/S0929139312001400

GC Conditions

GC instrument	Shimadzu GC-2010 Plus
Autosampler	Shimadzu AOC-20i Autoinjector and AOC-20S Autosampler
Software	MIDI Sherlock Software v.6.3B with PLFA Package (PLFAD2 v.2.00) Shimadzu LabSolutions v.5.85
Column	J&W Ultra 2, 25m x 0.2mm x 0.33µm film thickness (MIDI p/n Column G)
Liner	Split liner for focusing (Shimadzu p/n 220-94766-00)
Syringe	10µL syringe, fixed needle (Shimadzu p/n 221-34618-00)
Inlet temperature	250°C
Carrier gas	Hydrogen, constant velocity, 47.7 cm/min
Oven program	190°C, 10°C/min to 285°C (9.5 min), 60°C/min to 310 °C (0.42 min),
Split ratio	30:1
Injection volume	2.0µL

FID

Temperature	300°C

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