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Automated Phospholipid Fatty Acid (PLFA) Analysis using MIDI's Sherlock PLFA Analysis Software

Application Note – Agriculture | 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 has the ability to 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 serve as useful biomarkers for different microbial groups. PLFA analysis is a widely-used technique for estimation of the total 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 this analysis is performed manually, and the analysis process is laborious and potentially error-prone.

MIDI Inc.'s Sherlock PLFA Analysis Software automatically names all 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 discernable 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 Sherlock PLFA analysis of a soil sample 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.

After the *PLFAD2* Method was selected from the available methods within the Sherlock Sample Processor, the correct method parameters were automatically loaded into the Agilent GC ChemStation 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.

The Sherlock PLFA Tools software was then used to calculate mole percent, scale the data to the ISTD, calculate the biomass (nmol/g) for each microbial type (customizable) and calculate key PLFA ratios (customizable). 52 fatty acids were identified and 97.47% of the peaks were named.

Sherlock PLFA Analysis – Raw Peak Identification Report

Method: PLFAD2 File: E132054.66C Sample ID: D-PEN-13-01(24-SOIL G=2
Created: 2/5/2013 12:19:21 PM

RT	Response	RFact	ECL	Peak Name	%
0.7232	1.656E+9	----	7.7257	SOLVENT PEAK	----
1.0529	567	----	9.5153		----
2.1600	3893	1.056	13.6119	14:0 iso	0.66
2.3211	3753	1.035	13.9997	14:0	0.63
2.5421	6077	1.014	14.4399	15:1 iso w6c	0.99
2.5648	1236	1.011	14.4853	15:4 w3c	0.20
2.5878	1838	1.010	14.5310	15:1 anteiso w9c	0.30
2.6308	36686	1.006	14.6167	15:0 iso	5.95
2.6777	25287	1.002	14.7103	15:0 anteiso	4.08
2.7523	931	0.996	14.8588	15:1 w6c	0.15
2.8240	2834	0.991	15.0015	15:0	0.45
2.8562	2300	----	15.0566		----
3.0527	1199	0.978	15.3930	16:1 w7c alcohol	0.19
3.1859	13682	0.971	15.6211	16:0 iso	2.14
3.2423	894	0.969	15.7176	16:0 anteiso	0.14
3.2719	11207	0.967	15.7684	16:1 w9c	1.75
3.3036	50107	0.966	15.8227	16:1 w7c	7.80
3.3573	26955	0.964	15.9146	16:1 w5c	4.19
3.4077	58996	0.962	16.0008	16:0	9.14
3.4396	2993	----	16.0501		----
3.5382	1000	----	16.2026		----
3.6817	57533	0.953	16.4245	16:0 10-methyl	8.83
3.7255	4193	0.952	16.4923	17:1 iso w9c	0.64
3.7523	3240	0.951	16.5338	17:1 anteiso w9c	0.50
3.7766	601	0.951	16.5713	17:1 anteiso w7c	0.09
3.8120	10652	0.950	16.6261	17:0 iso	1.63
3.8735	10902	0.948	16.7213	17:0 anteiso	1.67
3.9231	4934	0.947	16.7980	17:1 w8c	0.75
3.9867	19667	0.946	16.8963	17:0 cyclo w7c	3.00
4.0562	3581	0.944	17.0036	17:0	0.54
4.0825	5951	0.944	17.0414	17:1 w7c 10-methyl	0.91
4.1286	1576	----	17.1077		----
4.3375	3504	0.940	17.4077	17:0 10-methyl	0.53
4.4001	1401	----	17.4978		----
4.4813	4062	0.939	17.6144	18:0 iso	0.61
4.5593	12731	0.938	17.7264	18:2 w6c	1.92
4.5916	51588	0.938	17.7729	18:1 w9c	7.80
4.6302	79549	0.938	17.8283	18:1 w7c	12.0
4.6954	11914	0.937	17.9221	18:1 w5c	1.80
4.7510	17661	0.937	18.0019	18:0	2.67
4.8111	6320	0.937	18.0850	18:1 w7c 10-methyl	0.95
4.8654	2513	0.936	18.1603	17:0 iso 3OH	0.38
5.0318	17852	0.936	18.3905	18:0 10-methyl	2.69
5.1131	759	0.936	18.5029	19:4 w6c	0.11
5.1541	1217	0.936	18.5597	19:3 w6c	0.18
5.2963	2156	----	18.7564		----
5.3379	1526	0.936	18.8140	19:1 w8c	0.23
5.4046	44722	0.936	18.9063	19:0 cyclo w7c	6.75
5.4748	13957	----	19.0033	19:0	----
5.6770	665	----	19.2768		----
5.7682	4816	0.938	19.4001	20:4 w6c	0.73
5.8238	2245	0.938	19.4753	20:5 w3c	0.34
5.8910	1338	0.939	19.5661	20:3 w6c	0.20
6.0414	6631	0.940	19.7695	20:1 w9c	1.00
6.2128	2615	0.941	20.0013	20:0	0.40
6.3548	2390	----	20.1937		----
6.7999	1502	0.946	20.7972	21:1 w8c	0.23
6.9162	1634	0.948	20.9549	21:1 w3c	0.25
7.1356	596	0.950	21.2533	22:5 w6c	0.09
7.6846	2087	0.955	22.0005	22:0	0.32
8.4044	837	0.960	22.9990	23:0	0.13
9.1112	2143	0.961	24.0007	24:0	0.33
9.4787	1784	----	24.5215		----

Total Response: 666023 Percent Named: 97.47% Peaks Named: 52

** 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.*

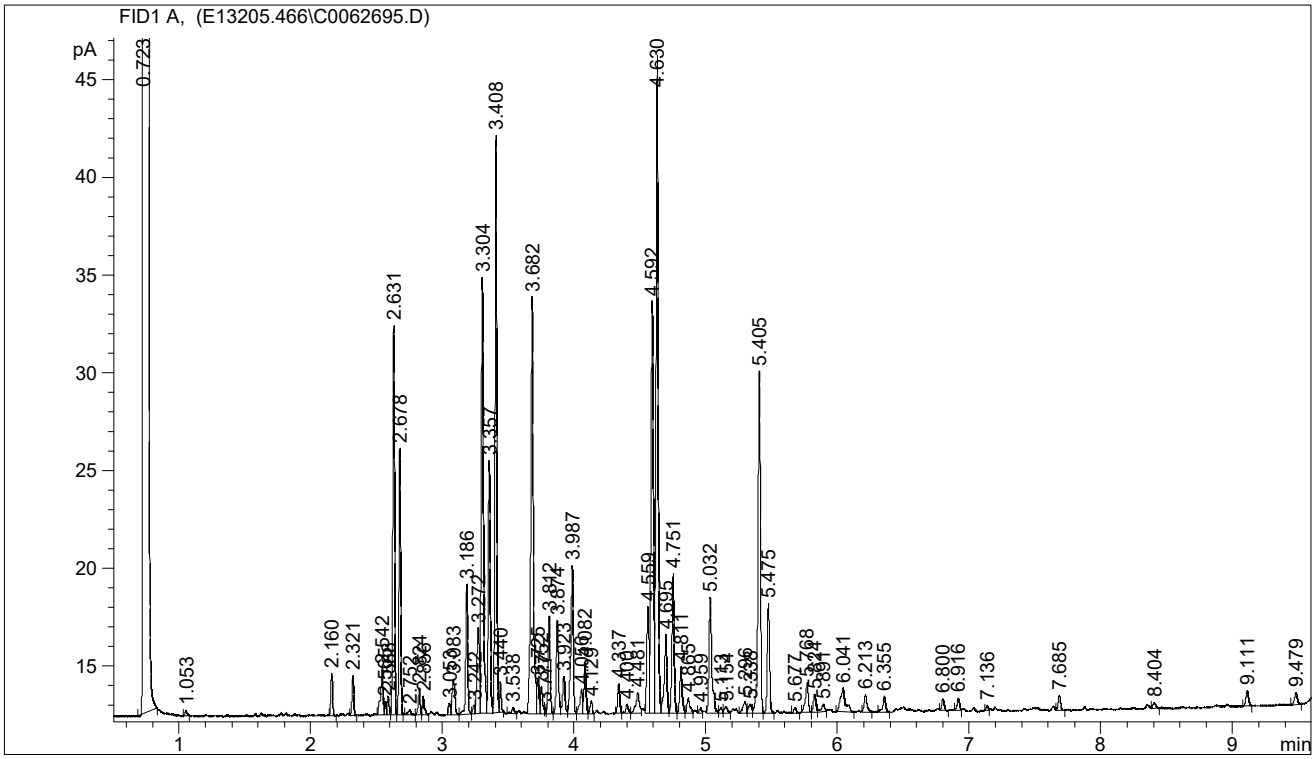

Figure 1. Representative chromatogram for analysis of the soil sample using the Agilent 7890B GC.

Sherlock PLFA Tools – Normalizes the data for molarity, Scales to an Internal Standard, Transforms the data into Biomass (nmol/g) & user-defined Ratios

Biomass (nmol/g) by Microbial Type

Method: MICSOIL3 File: E132054.MIC
Sample ID: D-PEN-13-01(24-SOIL G=2
Created: 9/24/2015 2:19:15 PM

PLFA Origin	Biomass (nmol/g)
Gram-Positive	33.21
Gram-Negative	56.30
AM Fungi	7.06
Fungi	2.96
Actinomycetes	21.54
Other Eukaryote	2.73
Not Assigned	35.93
Total PLFA	159.73

Key PLFA ratios calculated automatically

Method: RATIO3A File: E132054.RAT
Sample ID: D-PEN-13-01(24-SOIL G=2
Created: 9/24/2015 2:27:22 PM

Ratio Name	Ratio
Fungi/Bacteria	0.10
Predator/Prey	0.03
Gram+/Gram-	0.98
Sat/Unsat	0.84
Mono/Poly	10.54
GNeg Stress	2.14

Conclusion

PLFA analysis of soil samples via the Sherlock PLFA Analysis Software and Agilent GC 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 standardized PLFA protocol that can be implemented by most soil science laboratories for detailed study of the soil microbiota. User-defined variables (e.g. which fatty acids to assign to which microbial group) 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	Agilent 7890B Series
Autosampler	Agilent 7683 Injector and sample tray
Software	MIDI Sherlock Software v.6.3 with PLFA Package Agilent OpenLab CDS ChemStation
Column	Agilent Ultra 2, 25 m x 0.2 mm x 0.33 µm film thickness (MIDI p/n Column G)
Liner	Split liner, silanized (MIDI p/n 1221)
Inlet temperatrure	250 °C
Carrier gas	Hydrogen, constant flow, 1.3 mL/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	2.0 µL

FID

Temperature	300 °C

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