Workshop Report

Powerful new molecular tools in soil microbial ecology: insights and utility for long-term studies

2006 Long-term Ecological Research (LTER) All Scientists Meeting, Estes Park, Colorado, September 20-23, 2006.

Workshop organizers: Richard Boone (University of Alaska Fairbanks); David Myrold and Stephanie Boyle (Oregon State University); Mary Beth Leigh (University of Alaska Fairbanks); and Christopher Schadt (Oak Ridge National Laboratory).

The 2-hr workshop "Powerful new molecular tools in soil microbial ecology: insights and utility for long-term studies" took place at the LTER All Scientists Meeting (ASM) in Estes Park, Colorado on September 20, 2006. The workshop was broken up into two 1-hr sessions: one hour of oral presentations on novel molecular techniques and a question-and-answer period during the second hour. The presenters and their topics were David Myrold: the cellular basis for recent molecular techniques, Stephanie Boyle: terminal restriction fragment length polymorphisms (T-RFLP) and Quantitative PCR (Q-PCR), Mary Beth Leigh: Stable Isotope Probing (SIP), and Christopher Schadt: Microarrays. Questions from the audience in the second hour focused on methodological issues and how the methods can be used to address ecological questions. Forty (40) people attended the workshop and included graduate students, research staff, and faculty. Workshop attendees received a reference list for the techniques described as well as a definition sheet for the various acronyms used in the field. The workshop generated no plans for proposals or other products, as the intent was to provide basic information on evolving molecular techniques. The workshop was well received by the attendees, generated good discussion during the second hour and after the session, and clearly met a need.

Abstract

Ecosystem ecologists have long sought methods to best examine the microflora that regulate soil nutrient dynamics. Until roughly a decade ago direct examinations of the microflora that regulate key metabolic processes was limited mainly to direct counts, cultures, and biomass determinations. Many microflora, however, cannot be isolated or cultured easily or at all, and physiological processes in culture generally do not reflect those in situ. The past decade has seen the development of molecular tools that identify the functional components of soil microbial communities that regulate the soil nutrient dynamics long studied by ecologists at LTER sites. These powerful new tools offer ecologists the opportunity to directly measure the control of soil processes at the microbial gene level and to identify members of microbial communities that are directly involved in key metabolic processes. Examples of some of the new techniques include (1) stable isotope probing (SIP), which uses labeled nucleic acids as biomarkers and allows linkage between community composition and metabolic function, and (2) quantitation of mRNA, which can be used to directly measure expression of microbial genes that regulate soil nutrient transformations. Although these new tools offer great promise to capture more directly the functional status of the soil microflora, most ecosystem ecologists are not well informed about the value of the tools, the technical procedures, and methodological limitations. Furthermore there has been insufficient discussion about how best to link molecular information about soil microflora to properties of interest at the ecosystem and landscape levels. The purpose of this working group is to provide an overview of the emerging microbial molecular techniques and to stimulate discussion on how these new approaches can be used to address questions and objectives of the LTER program.

REFERENCES FOR METHODS

T-RFLP and Q-PCR

Hackl, E., S. Zechmeister-Boltenstern, L. Bodrossy, A. Sessitsch. 2004. Comparison of diversities

and compositions of bacterial populations inhabitting natural forest soils. Applied Environ Microb 70:5057-5065.

Rösch, C., H. Bothe. 2005. Improved assessment of denitrifying, N2-fixing, and total-community bacteria by terminal restriction fragment length polymorphism analysis using multiple restriction enzymes. Applied Environ Microb 71:2026-2035.

Okano, Y., K.R. Hristova, C.M. Leutenegger, L.E. Jackson, R.F. Denison, B. Gebreyesus, D. Lebauer, K.M. Scow. 2004. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. Applied Environ Microb 70:1008-1016.

Stubner, S. 2002. Enumeration of 16S rDNA of Desulfotomaculum lineage 1 in rice field soil by real-time PCR with SybrGreenTM detection. J Microb Methods 50:155-164.

Fierer, N., J.A. Jackson, R. Vilgalys, R.B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied Environ Microb 71:4117-4120.

Microarrays

Brodie, E. L., T. Z. DeSantis, D. C. Joyner, S. M. Baek, J. T. Larsen, G. L. Andersen, T. C. Hazen, P. M. Richardson, D. J. Herman, T. K. Tokunaga, J. M. Wan, and M. K. Firestone. 2006. Application of a High-Density Oligonucleotide Microarray Approach To Study Bacterial Population Dynamics during Uranium Reduction and Reoxidation. Appl. Environ. Microbiol. **72:**6288-6298.

Gentry, T. J., G. S. Wickham, C. W. Schadt, Z. He, and J. Zhou. 2006. Microarray applications in microbial ecology research. Microbial Ecology. 52:159–175.

Liebich, J., C. W. Schadt, S. C. Chong, Z. L. He, S. K. Rhee, and J. Z. Zhou. 2006. Improvement of oligonucleotide probe design criteria for functional gene microarrays in environmental applications. Applied and Environmental Microbiology **72:**1688-1691.

Rhee, S. K., X. D. Liu, L. Y. Wu, S. C. Chong, X. F. Wan, and J. Z. Zhou. 2004. Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Applied and Environmental Microbiology **70:**4303-4317.

Wu, L., X. Liu, C. W. Schadt, and J. Zhou. 2006. Microarray-Based Analysis of Subnanogram Quantities of Microbial Community DNAs by Using Whole-Community Genome Amplification. Appl. Environ. Microbiol. **72:**4931-4941.

Stable Isotope Probing

Evershed, R.P., Z.M. Crossman, I.D. Bull, H. Mottram, J.A. Dungait, P.J. Maxfield, and E.L. Brennand. 2006. 13C-Labelling of lipids to investigate microbial communities in the environment. Curr. Opin. Biotechnol. 17:72-82.

Friedrich, M. W. 2006. Stable-isotope probing of DNA: insights into the function of uncultivated microorganisms from isotopically labeled metagenomes. Curr. Opin. Biotechnol. 17:59-66.

Madsen, E. L. 2006. The use of stable isotope probing techniques in bioreactor and field studies on bioremediation. Curr. Opin. Biotechnol. 17:92-97.

Prosser, J. I., J. I. Rangel-Castro, and K. Killham. 2006. Studying plant-microbe interactions using stable isotope technologies. Curr. Opin. Biotechnol. 17:98-102.

Whiteley, A. S., M. Manefield, and T. Lueders. 2006. Unlocking the `microbial black box' using RNA-based stable isotope probing technologies. Curr. Opin. Biotechnol. 17:67-71.

Abbreviations

AFLP - amplified fragment length polymorphism
ALFA - automatic laser fluorescence analysis
AP-PCR - arbitrarily primed PCR
ARDRA - amplified ribosomal DNA restriction analysis
ARISA - automated ribosomal intergenic spacer analysis
BAC - bacterial artificial chromosome
BLAST - Basic Local Alignment Search Tool
BrdU - bromodeoxyuridine
cDNA - coding DNA (reverse transcribed RNA)
Cy5/Cy3 - cyanine based florescent dye (used to label DNA or RNA)
DAF - DNA amplification fingerprinting
DD - differential display
DGGE - denaturing gradient gel
ERIC - enterobacterial repetitive intergenic consensus
FASTA - Fast All
FERP - fluorescent-enhanced rep-PCR
FGA functional gene microarray
FISH - fluorescent in situ hybridization
GFP - green fluorescent protein
IEF - isoelectric focusing
IGS - inter-ribosomal gene spacer sequence
ISR - intergenic spacer region polymorphism
ITS - internal transcribed spacer
ITS-PCR-RFLP - inter-transfer ribosomal-PCR-RFLP
LH-PCR - length heterogeneity PCR
MDA - multiple displacement amplification (phi29 polymerase amplification of linear DNA templates)
MGE - mobile genetic elements
MLST - multi-locus sequence typing
OTU - operational taxonomic unit
PCR - polymerase chain reaction
PFGE - pulsed field gel electrophoresis
PLFA – phospholipids fatty-acid analysis
PNA - peptide nucleic acid
qPCR - quantitative real-time PCR
RAPD - random amplified polymorphic DNA
RAP-PCR - RNA fingerprinting by arbitrarily primed PCR
RCA - rolling circle amplification (phi29 polymerase amplification of circular DNA templates)
rDNA - ribosomal DNA

REP - repetitive extragenic palindromic sequence
rep- PCR - repetitive sequence-based PCR
RFLP - restriction fragment length polymorphism
RSGP - reverse sample genome probing electrophoresis
RT - reverse transcription
SIG-PCR - signature PCR
SIP - stable-isotope probing
SSCP - single strand conformation polymorphism
TGGE - temperature gradient gel electrophoresis
T-RLFP - terminal restriction fragment length polymorphism
UPGMA - unweighted pair group method with arithmetic mean
VNTR - variable number of tandem IS insertion sequences repeat

AMF - arbuscular mycorrhizal fungi
AOB - ammonia-oxidizing bacteria
BSA - bovine serum albumin
CAT - chloramphenicol acetyl
CTAB - hexadecyltrimethylammonium bromide
ddNTP 2' ,3' - dideoxynucleoside
DEPC - diethylpyrocarbonate
dNTP - deoxynucleoside triphosphate
EDTA - ethylenediamine tetraacetic acid
IPTG - isopropyl-β- D- thiogalactopyranoside
MDH - methanol dehydrogenase
MMO - methane monooxygenase
PBS - phosphate-buffered saline
PFA - paraformaldehyde
SDS - sodium dodecyl sulfate
UV - ultraviolet

LIST OF PARTICIPANTS

Date of Working Group: September 20, 2006

	NAME	LTER Site or Affiliation	Email address
1.	Rich Boone	Bonanza Creek (BNZ)	r.boone@uaf.edu
2.	David Myrold	Andrews (AND)	David.Myrold@oregonstate.edu
3,	Stephanie Boyle	Andrews (AND)	stephanie.boyle@oregonstate.edu
4.	Chris Schadt	Oak Ridge National Lab, DoE	schadtcw@ornl.gov
5.	Mary Beth Leigh	Bonanza Creek (BNZ)	ffmbl@uaf.edu
6.	Brian Hopkinson	CCE, Scripps Institution of Oceanography	bhopkins@ucsd.edu
7.	Anita Antoninka	CCR, Northern Arizona University	ajac@nau.edu
8.	Barbara Bahnmann	Kellogg (KBS), University of Western Ontario, University of Alberta	barbara.bahnmann@telus.net
9.	Lydia Zeglin	Sevilleta (SEV), University of New Mexico	lzeglin@unm.edu
10.	Luis M. Garcia-Ort	Luquillo (LUQ), University of Turabo	luismanuell1@aoc.com
11.	Sharon Cantrell	Luquillo (LUQ), University	scantrel@suagm.edu

		of Turabo	
12.	Gwyneth Gordon	SESE, Arizona State University	gwyneth.gordon@asu.edu
13.	Melany Fisk	Hubbard Brook (HBR), Appalachian State University	fiskmc@appstate.edu
14.	Grizelle Gonzalez	Luquillo (LUQ), USFS, Puerto Rico	ggonzalez@fs.fed.us
15.	Kurt Thelen	Michigan State University	thelenk3@msu.edu
16.	Ari Jumpponen	Konza Prairie LTER, Kansas State University	ari@ksu.edu
17.	Kristen Freeman	Niwot Ridge LTER	kristen.freeman@colorado.edu
18.	Craig Judd	LTER Short Grass Steppe, Colorado State University	Craig.Judd@colostate.edu
19.	Andrew King	Niwot Ridge LTER	kingaj@colorado.edu
20.	Matt Wallenstein	Arctic LTER, Harvard University, Colorado State University	mawallen@nrel.colostate.edu
21.	Becky Ball	Coweeta LTER, University of Georgia	bball@uag.edu
22.	Stacie Kageyama	Andrews (AND), Oregon State University	Stacie.Kageyama@oregonstate.edu
23.	Ben Wolfe	Harvard Forest, Harvard University	bwolfe@oeb.harvard.edu
24.	Kat Mclain	Konza LTER	schmitt@ksu.edu
25.	Alix Contosta	Harvard Forest, University of New Hampshire	alix.contosta@unh.edu
26.	Jeb Barrett	McMurdo Dry Valleys	John.E.Barrett@Dartmouth.edu
27.	Marie Sabacka	McMurdo Dry Valleys	marie.sabacka@myportal.montana.edu
28.	Brianna Arnold	MCM	brianna.arnold@myportal.montana.edu
29.	Kristen M.S. Myers	Palmer	KMSMyers@vims.edu
30.	Tom Schmidt	Kellogg Biological Station	tschmidt@msu.edu
31.	Caroline Bledsoe		
32.	Chuck Rice	Konza LTER	

8 other participants were unknown and did not sign the attendance sheet.