

Molecular analysis of microbial community structure

Two underlying views of the Microbial communities: Functional vs. Organismal (Ecosystems vs. ecological approach)

typical functions that one might measure: C fixation and cycling : "NPP" net primary productivity, respiration; nutrient cycling / retention N fixation; biodegradation of particular substrates.

Questions about microbial communities

What is the structure: species present, abundance
"diversity" = richness + evenness (Ranked abundance curves, and effort curves)

Who is active and what do they do?

Does this structure change with disturbance? if so how?

Does the structure effect the function?

What do individual species do in the community? - are they "functionally redundant"

How can so many species co-exist?

Can we manipulate communities to improve a given function (e.g. Bioremediation)

Can we identify useful gene products within these communities

Total community characterization w/o identification

Biolog - based on sole carbon source growth and tetrazolium color change (Campbell et al., 1997; Hitzl et al., 1997; Insam, 1997, <http://www.biolog.com/>)
only aerobic culturables - primarily fast growers,
many substrates irrelevant - but improved in "eco biolog"
reproducibility a problem - inoculum density and incubation time critical
Comparisons by PCA-like analyses (Principle components) (Hitzl et al., 1997)

FAME (Fatty acid methyl ester) and PLFA (Phospholipid fatty acids) profiles (Ludvigsen et al., 1997)

base on gas chromatograph of extracted lipids and fatty acids, FAME profiles include dead and inactive components, PLFA primarily active living membrane fraction

Sequence based methods coupled with PCR

Targets for sequencing

SSU (16 & 18S) rRNA - used mostly with prokaryotes

3 Properties that make it useful - "universal primers", copy # particularly of RNA, database <http://rdp.cme.msu.edu/index.jsp>

Protein coding loci (Santos and Ochman 2004). - scan completed genomes for universal proteins that have regions conserved enough for primer design. Primers for ten candidate genes produced. 60% success rate in amplifying targets.

approach

Basic method: amplify, clone, screen, sequence

Artifacts: chimeric sequences, minor sequence errors; multiple non-identical copies of gene; DNA present but organism isn't, Organism is present but inactive

Interpretation of close matches and distant matches

PCR-based methods

biases: extraction: gram positive, spores, small size

amplification: high G+C, priming sequences, introns, copy number, operon structure, numerical biases

ratios amplified - differ with primers used, eventually all are equal

cloning biases - blunt versus sticky end different results in one study

Errors - misincorporation, chimeras

Other problems (not really PCR related)

Intrastrain and intra-operon difference

SSU "fingerprints"

DGGE (denaturing Gradient Gel Electrophoresis), & TGGE (temperature gradient) - "quasi-quantitative" (Muyzer & Smalla, 1998), SSCP Schmalenberger & Tebbe 2003

T-RFLP analysis of 16S (Moeseneder et al., 1999), and ARDRA (amplified rDNA restriction analysis)

Reasonably semi-quantitative according to [Lueders, 2003 #6153]
according to Moeseneder is more sensitive than DGGE

ribosomal internal spacer (RISA) amplification (Garcia-Martinez et al., 1999)

Quantification via oligonucleotide probing

hybridization to filters with blotted rRNA (Stahl fig.)

Specific hybridization/universal
what about unknown probe specificities?

FISH florescent in situ hybridization with visual counts (Amann, 1995)

Targeted at rRNA

fixation method important - usually alcohols or aldehydes, some gram

positive require lytic enzymes or organic solvents

some sites on in situ rRNA not accessible to probes

simultaneous double probing to check result

Problems with soil: autofluorescence of organic in soil, <1% of cells show hybridization in some studies, irregular distribution of cells and adhesion to particles

Quantification via realtime PCR - based on measure product as it accumulates (see Mackay et al 2002 or Sharkey et al., 2004) for a review),(Landeweert et al 2003 paper for Thursday)

simplest way involves intercalary dyes (ethidium, SYBER green, YO-PRO-1) - accumulation of double-stranded product detected.

accumulation of specific product can be monitored with specific probes

this depends on **FRET**: fluorescence resonance energy transfer

either two fluorescent labels or one fluorescent label and a **NFQ** (non-fluorescent quencher)

several variations (more all the time):

5'nuclease oligoprobes or "TaqMan" probes

linear probes stable probes

hairpin probes (or "molecular beacons")

SNIP monitoring via melt profile

Microarrays – (Wilson et al 2002)

60,000 probes/chip - hybridize fluorescently labeled PCR pool, or cDNA pool

May miss total unknowns

Reading differences in hybridizations within array may be tricky

Currently expensive

Approaches unique to fungal communities -

Collections of fruiting bodies for macrofungi

lots of studies, in ectomycorrhizal communities bad correlation with below ground

Culture for wood decay communities and saprobic communities - soils yield primarily fast growing ascomycetes, modifications for Basidiomycetes
 particle filtration method for litter (Polishook et al., 1996)
 yields 134 to 228 species/litter
 relation to pharmaceutical co.

Molecular

O'Brian et al (2005) first study to look at soil fungi, most studies focus on root colonizing symbionts (mycorrhizae) - avoids quantification problems

ITS-RFLP very common in ectomycorrhizal communities
 (quantification by root tip)

Low (but real) intraspecific variation

Very high interspecific variation

Some patterns can be matched to species, most remain unidentified

ITS Sequence database very deficient, but 5.8S is useful for placement at phylum level (Ascomycota vs. Basidiomycota) (Cullings and Vogler, 1998)

18S sequence - largest use in AM mycorrhizal communities (Helgason et al., 1999)

Basidiomycetes resolution may be too low

mt-LSU partial sequence places most ectomycorrhizal basidiomycetes into major groups (Bruns et al., 1998), nuc-LSU now even more extensive (Moncalvo et al 2002)

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- read for discussion on Tuesday