Analysis of bacterial community structure in soil microcosms

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Methods for analysis of bacterial community structure in soil microcosms

Methods

Several methods are available for the study of microbial diversity in soil.

The methods can be broadly divided into two groups:

• **culture-dependent methods**
  methods that require growth or activity e.g. colony morphology from plate counts and community level physiological profiling (CLLP) using Biolog plates

• **culture-independent methods**
  primarily methods using PCR-amplification of nucleic acids
Methods for analysis of bacterial community structure in soil microcosms

Different methods reflect different aspects of bacterial diversity or community structure. All methods suffer from various limitations. No single method reflects the "true" diversity or species composition.

<table>
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<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Culture-dependent methods (plate counts, CLPP)</td>
<td>Possible to obtain knowledge about identity and physiological or functional properties of organisms detected</td>
<td>Only culturable organisms included</td>
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<td></td>
<td>Inexpensive, no special equipment needed</td>
<td>Fast growing organisms are favoured</td>
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<tr>
<td>Molecular fingerprinting techniques (eg. DGGE, LH-PCR, T-RFLP)</td>
<td>Also non-culturable organisms can be detected</td>
<td>Bias due to PCR, differences in extraction efficiency etc.</td>
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<td>Many samples can be analyzed simultaneously.</td>
<td>Bands or peaks can represent several different species</td>
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Often useful to use several techniques!
Methods for analysis of bacterial community structure in soil microcosms

Methods

There are no principal differences in the applicability of methods in microcosm studies and in e.g. field studies. This presentation is not intended as a comprehensive overview of available methods for the study of microbial diversity (these methods has been reviewed several times e.g. Kirk et al. 2004. *Journal of Microbiological Methods* 58: 169-188).

Instead we will give some examples on how different methods can be applied in soil microcosm studies and how different techniques can be combined to increase the amount of information and taxonomic resolution.
The use of soil microcosms in studies of bacterial communities

Soil microcosms are useful for investigations of the effect of various environmental or biological factors on bacterial communities.

Soil microcosms have several advantages, e.g.:

- variability between replicates can be strongly reduced compared to field studies

- environmental factors can be manipulated in a consistent and reproducible way

Furthermore, by using microcosms with previously sterilised soil it is possible to manipulate diversity in the microcosms in various ways. The sterilised soil can be reinoculated with different organism groups. This allows specific hypotheses about the impact of biotic interactions on microbial diversity to be tested.
Microbial diversity in soil microcosms evaluated by molecular fingerprinting

The combination of soil microcosms with molecular fingerprinting techniques can be very useful.

- Microcosms can be set up with sieved soil reducing the heterogeneity usually found in the field.
- Molecular fingerprinting techniques allow many replicates to be performed simultaneously with relatively high reproducibility.

This means that this experimental setup is very useful for investigations of the relative importance of different environmental factors for the bacterial community structure. It allows important factors to be identified

– **BUT** the molecular fingerprinting techniques do not give any direct information about which organisms respond to a given treatment – only that there is an effect on the community structure.
Microbial diversity in soil microcosms evaluated by molecular fingerprinting

In the following we will give some examples on how molecular fingerprinting techniques can be used in soil microcosm studies

- and how they can be combined with other techniques to provide more detailed information about which organisms actually respond to a given treatment.
Use of Denaturing Gradient Gel Electrophoresis (DGGE) to assess the impact of protozoan grazing on bacterial community structure.

Soil microcosm experiment. Sterilised soil reinoculated with soil bacteria (-P) or with bacteria and protozoa (+P)

Two different substrates added (ground roots or repeated additions of an artificial root exudate to mimic the rhizosphere environment)

Bacterial community structure assessed by DGGE, colony morphology from plate counts and CLPP (Biolog plates)
Denaturing Gradient Gel Electrophoresis (DGGE)

1. DNA extracted from soil


3. PCR products loaded on a denaturing gel

4. Depending on sequence and GC content the fragments migrate to different positions in the gel (the GC-clamp prevents complete denaturation). Note that fragments from distantly related organisms may co-migrate to the same position in the gel.
Effect of protozoan grazing on bacterial community structure - determined by DGGE

Bands decreasing in intensity with grazing

Bands increasing in intensity with grazing

The DGGE banding patterns showed clear effects on bacterial community structure – both of protozoan presence and type of substrate – but it gives no information about which bacteria responded

Excision of bands

On the gel shown on the previous slide the bands were visualised by silver-staining. This precludes reamplification of DNA from excised bands but the same samples were run on a similar gel that was stained by ethidium bromide. The bands on this gel were visualised under UV-illumination and selected bands were excised.

DNA was extracted, reamplified and sequenced (in some cases the bands contained PCR-products from different bacteria and it was necessary to clone). The sequences were compared with sequences in GenBank.
Effect of protozoan grazing on bacterial community structure
- bands identified by sequencing -

- Roots
- Nutrient solution

-P +P -P +P

Bdellovibrio (δ-proteobacteria, G-)
Buttiauxella (γ-proteobacteria, G-)
Bdellovibrio (δ-proteobacteria, G-)
Leifsonia (Actinobacteria, G+)
Achromobacter (β-proteobacteria, G-)
Arthrobacter (Actinobacteria, G+)
Alcaligenes (β-proteobacteria, G-)

Correlation between molecular fingerprinting and culture-dependent techniques

Diversity indices were calculated based on molecular fingerprinting data (band presence and intensity on DGGE gels) and on data for the culturable bacteria (colony morphology from plate counts)

![Graph showing correlation between Shannon index ($H'$) and sequential sampling index. The graph has a trend line with $R^2 = 0.85$, $P < 0.001$.](image)
Correlation between molecular fingerprinting and culture-dependent techniques

In this case there is good agreement between measures of diversity obtained for the culturable fraction and for the extracted DNA. This is probably due to special features of this experiment: The organisms were regrown from a small inoculum. This has favoured fast-growing (and presumably culturable) organisms.

In other cases there may be lower correlation between the measures obtained by different methods.
The possibility of excising and sequencing bands from DGGE gels is one of the advantages of DGGE compared to other molecular techniques such as LH-PCR (length heterogeneity PCR) and T-RFLP (Terminal Restriction fragment length polymorphism).

However, these techniques have other advantages: the fingerprinting patterns obtained are more generally reproducible. The patterns obtained consist of peaks representing different fragment lengths and this length is well-defined so it is possible to compare patterns between different studies.
Combining LH-PCR with analysis of single bacterial isolates

Length heterogeneity PCR (LH-PCR)

1. DNA is extracted from soil

2. Fragments of the 16S rRNA gene are amplified (eg using the universal eubacterial primers BSF8/20 and BSR534/18). One of the primers is fluorescently labelled.

3. The PCR products are run on a sequence gel.

Combining LH-PCR with analysis of single bacterial isolates

LH-PCR does not allow bands to be excised and sequenced. However, the length of the fragments can give an indication of the taxonomic identity of the organism. Furthermore, bacteria can be isolated from the soil investigated. These isolates can be tentatively identified by sequencing and fragment length determined. The figure below shows the LH-PCR pattern of an isolate and of the total community in a soil. A large proportion of the isolates examined belonged to the α-proteobacteria and peaks with fragment lengths corresponding to α-proteobacteria also dominated in the LH-PCR patterns.
Comparison of selected aspects of DGGE, T-RFLP and LH-PCR

Bias due to DNA extraction, PCR, operon copy number, single cell variability in 16S rRNA gene sequence etc. are similar for the three techniques

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<th>Resolution (ability to discriminate between species)</th>
<th>Reproducibility between gels</th>
<th>Identification of relevant OTU’s</th>
<th>Specific advantages</th>
<th>Problems</th>
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<tbody>
<tr>
<td>DGGE (eg. 2)</td>
<td>Potentially high. Depends on the community investigated</td>
<td>Low (fragments may migrate different distances in different gels)</td>
<td>Direct by excising and sequencing bands</td>
<td></td>
<td>Co-migration of sequences from phylogenetically unrelated organisms to same band position</td>
</tr>
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<td>T-RFLP (eg. 1, 2, 3, 4)</td>
<td>Potentially high. Depends on the community investigated and the restriction enzymes used</td>
<td>High (Fragments are characterized by absolute lengths)</td>
<td>Indirect by comparing fragment lengths with clones or isolates</td>
<td>Fragment length gives distinct and reproducible information that can be related to taxonomic identity</td>
<td>Unrelated organisms may give similar fragment lengths</td>
</tr>
<tr>
<td>LH-PCR (eg. 1, 3)</td>
<td>Potentially high. Depends on the community investigated. Taxa from some broad taxonomic groups may have similar fragment lengths</td>
<td>High (Fragments are characterized by absolute lengths)</td>
<td>Indirect by comparing fragment lengths with clones or isolates</td>
<td>Fragment length can be related to taxonomic identity (as for T-RFLP)</td>
<td>Unrelated organisms may give similar fragment lengths</td>
</tr>
</tbody>
</table>

References:
Conclusions

• The combination of soil microcosms with molecular fingerprinting techniques provides a reproducible and useful way of investigating effects of various abiotic and biotic parameters on bacterial community structure

• By combining different techniques more detailed information and higher taxonomic resolution can be obtained