



**DGGE technique and its application in
marine environmental microbial diversity study**

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Outline

1 Marine microbes

2 Principle of DGGE

3 Key steps and optimization

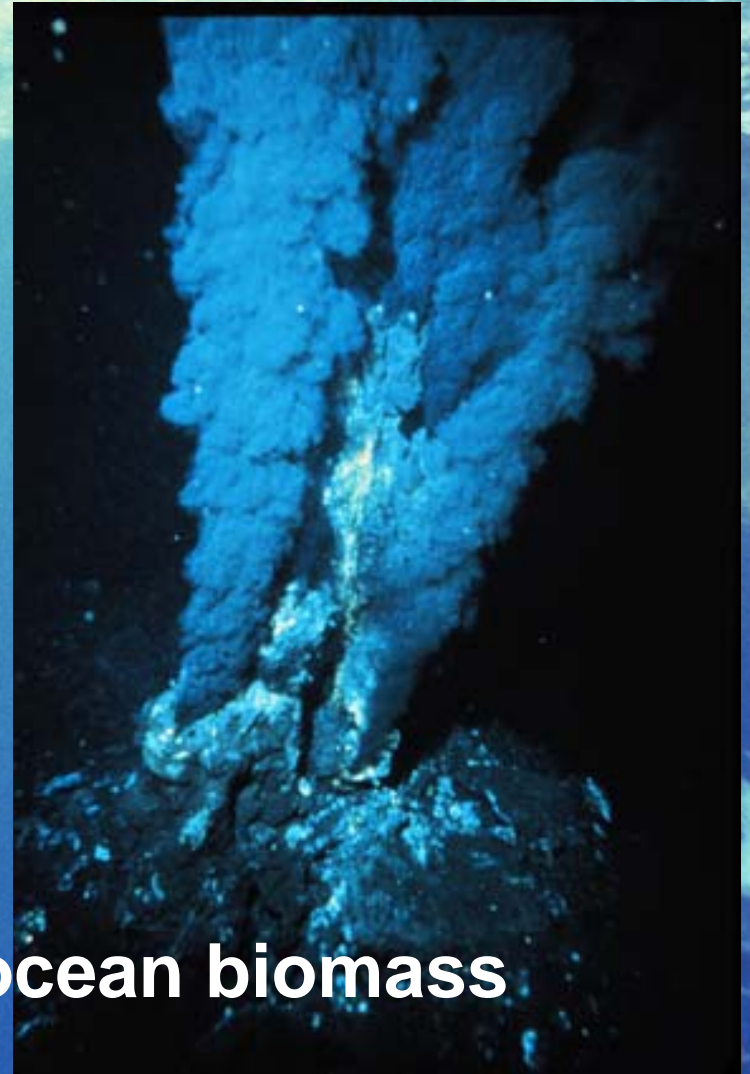
4 Application in marine microbial diversity

5 Development of DGGE



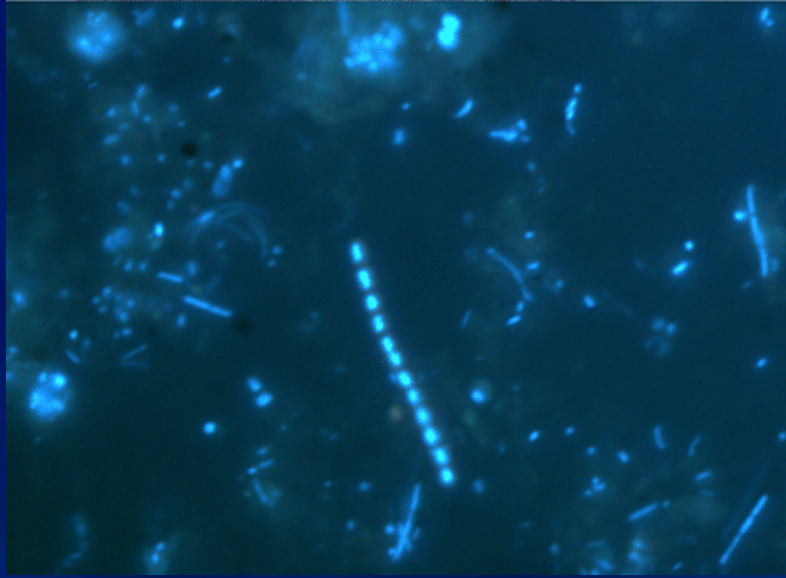
The Blue Planet

Marine microbes



account for more than 90% of ocean biomass

Play crucial roles



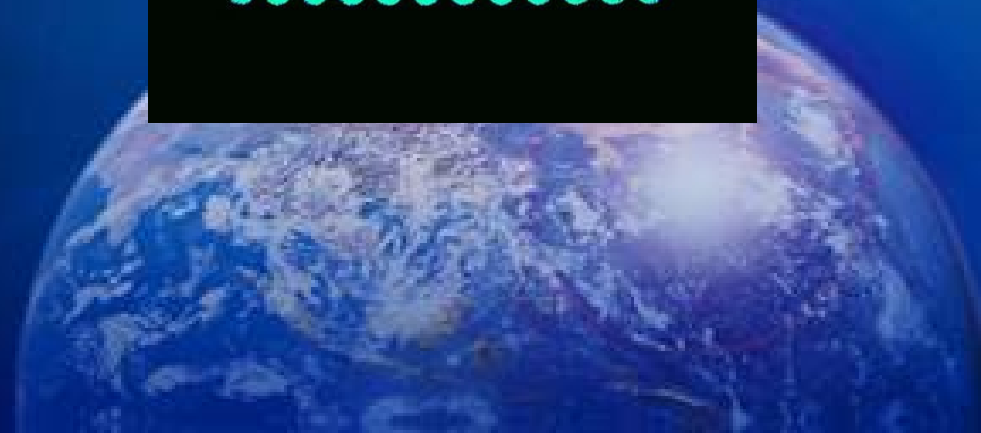
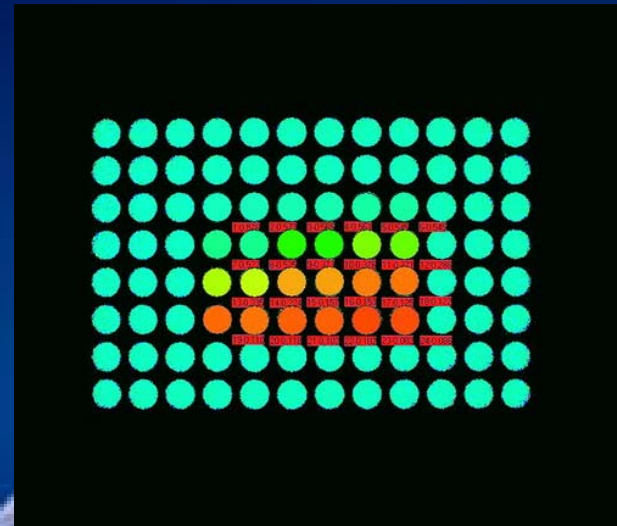
- **Decomposition of organic matter**
- **Cycling of nutrients and carbon**
- **Microbial pathogens**
- **Bioremediation capabilities**



Research approaches

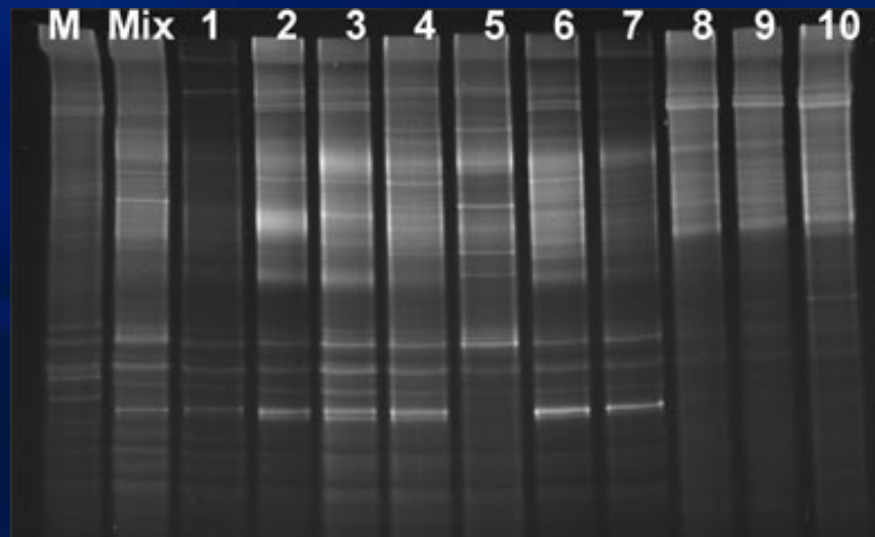
Traditional cultivation

Molecular technique



D G G E

Denaturing gradient gel electrophoresis (DGGE) is a molecular fingerprinting method that separates PCR products.



Introduced in microbial ecology by Muyzer in 1993

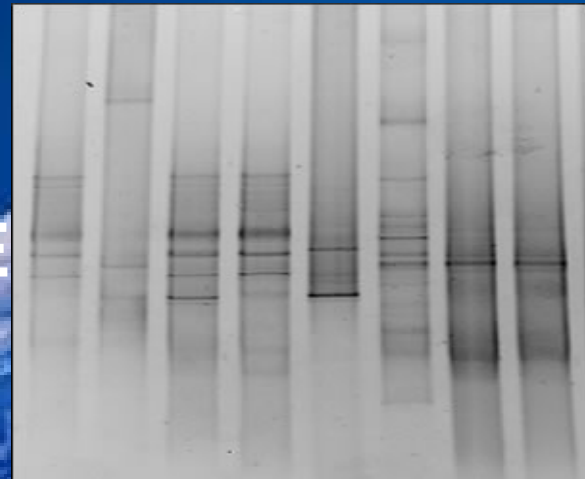
Principle of DGGE

DGGE can separate PCR products based on sequence differences. It is the mobility shift which can differentiate slightly different sequences. Each band theoretically represents a different bacterial population present in the community.

PCR



DGGE



Key steps and optimizations

Essentially, PCR-DGGE comprises three steps:

- A** Extraction of total community DNA from the samples
- B** PCR amplification
target sequences: 16S rDNA, 18S rDNA
GC clamp
- C** Separation of the PCR products using DGGE



Protocol in brief of DGGE separation



Caution

DGGE gels will be prepared by combining two solutions containing acrylamide and denaturants (urea and formamide) to form a gradient of denaturant. Wear gloves during all steps and change them when contamination is even suspected !



Assemble the gel sandwich

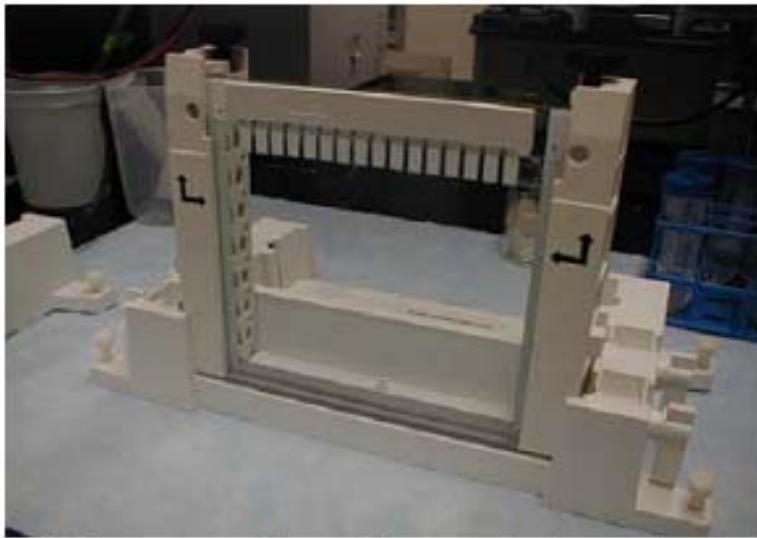
Components of the gel sandwich:

two glass plates, gel clamps, spacers and combs

Clean any of the components thoroughly before assembling and clamps must be tight enough to prevent leakage.



Running the gel



Gel assembly with comb inserted.



The DGGE apparatus in action.

Take care that **no air bubbles** under the comb teeth. Load appropriate amounts of PCR products and begin to electrophorese for 16 h at 100 V (or 4 h at 200 V).

Staining the gel

When the electrophoresis is complete, the gel will be stained to reveal band patterns that can be used to determine the similarity of sampled microbial communities.

Ethidium bromide, EB

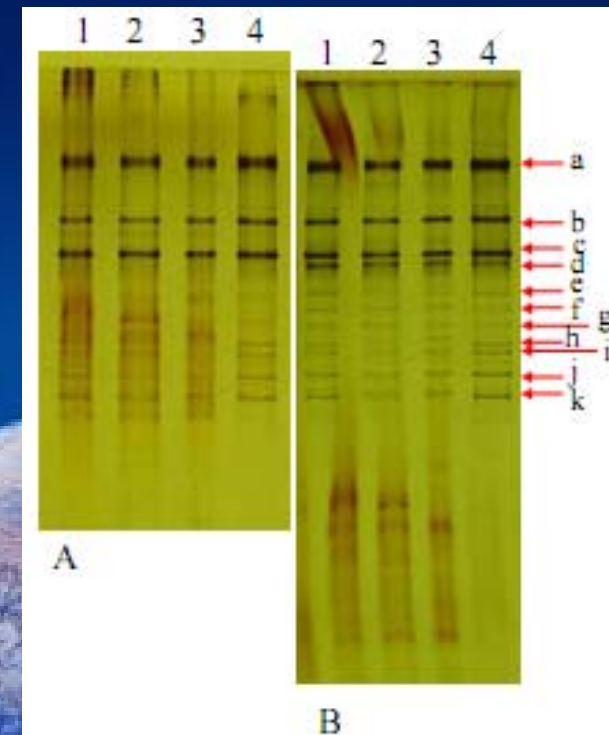
SYBR Green I

SYBR Gold

Silver staining



more sensitive



Case study 1

Diversity of marine picoeukaryotic assemblages

DNA extraction



PCR amplification

18S rDNA universal primer



DGGE and cluster analysis

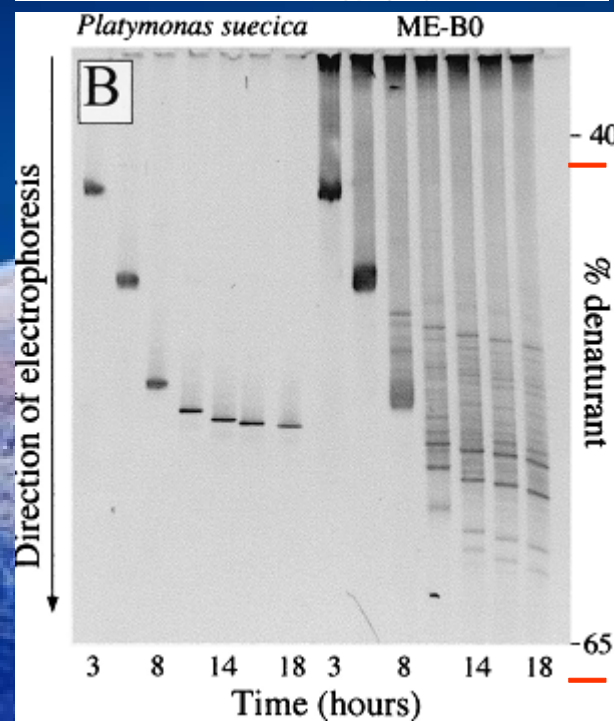
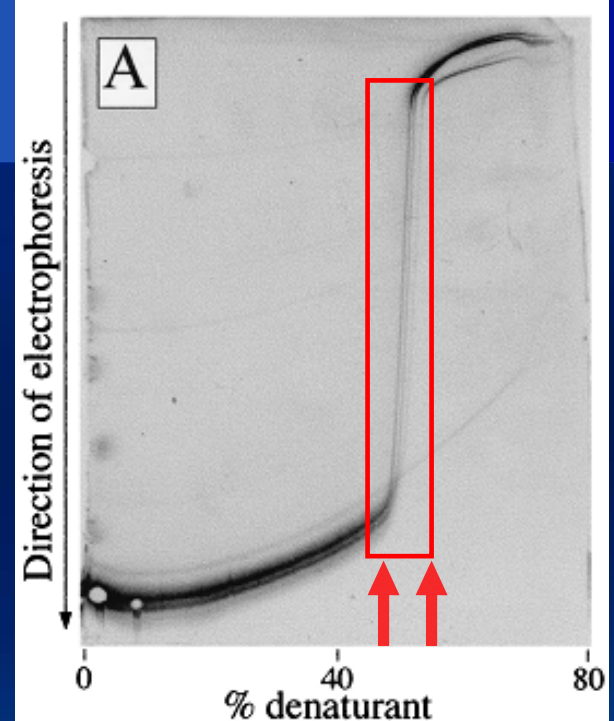


Optimization of gradient

A perpendicular DGGE analysis was performed to determine an appropriate gradient of denaturant concentrations.

At a denaturant concentration range of 50% to 55%, the fragments displayed reduced mobility.

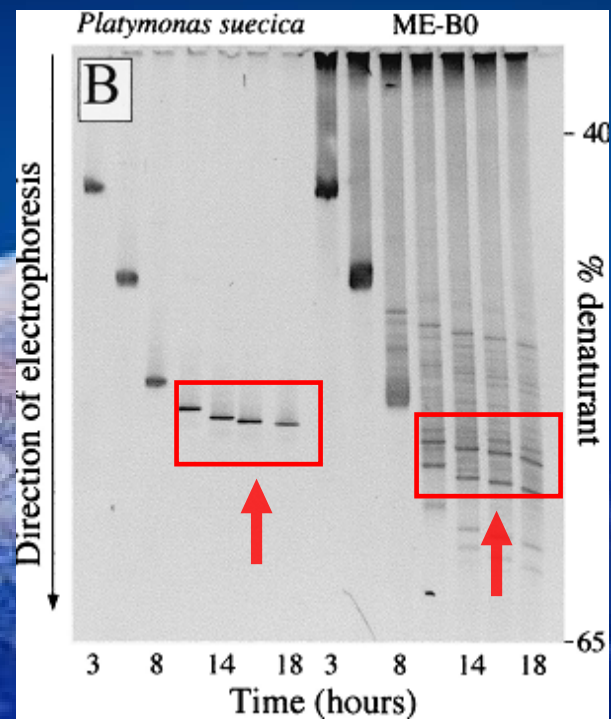
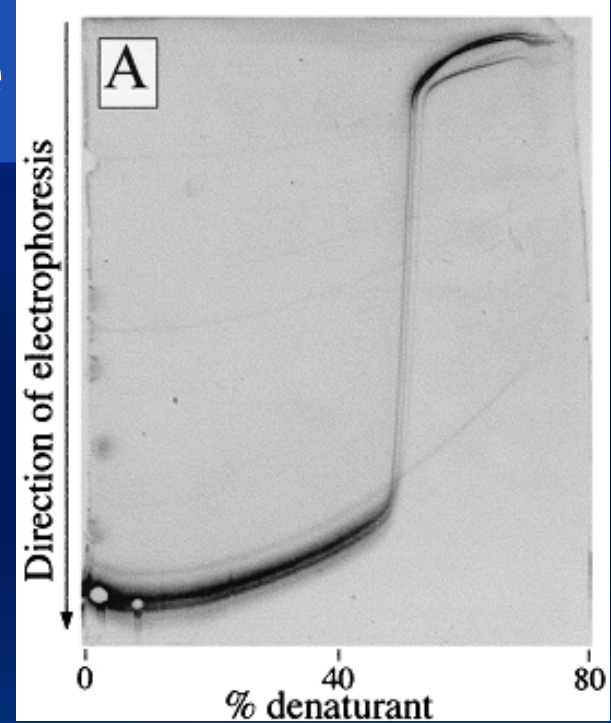
So the optimal denaturant gradient was 40% to 65%.

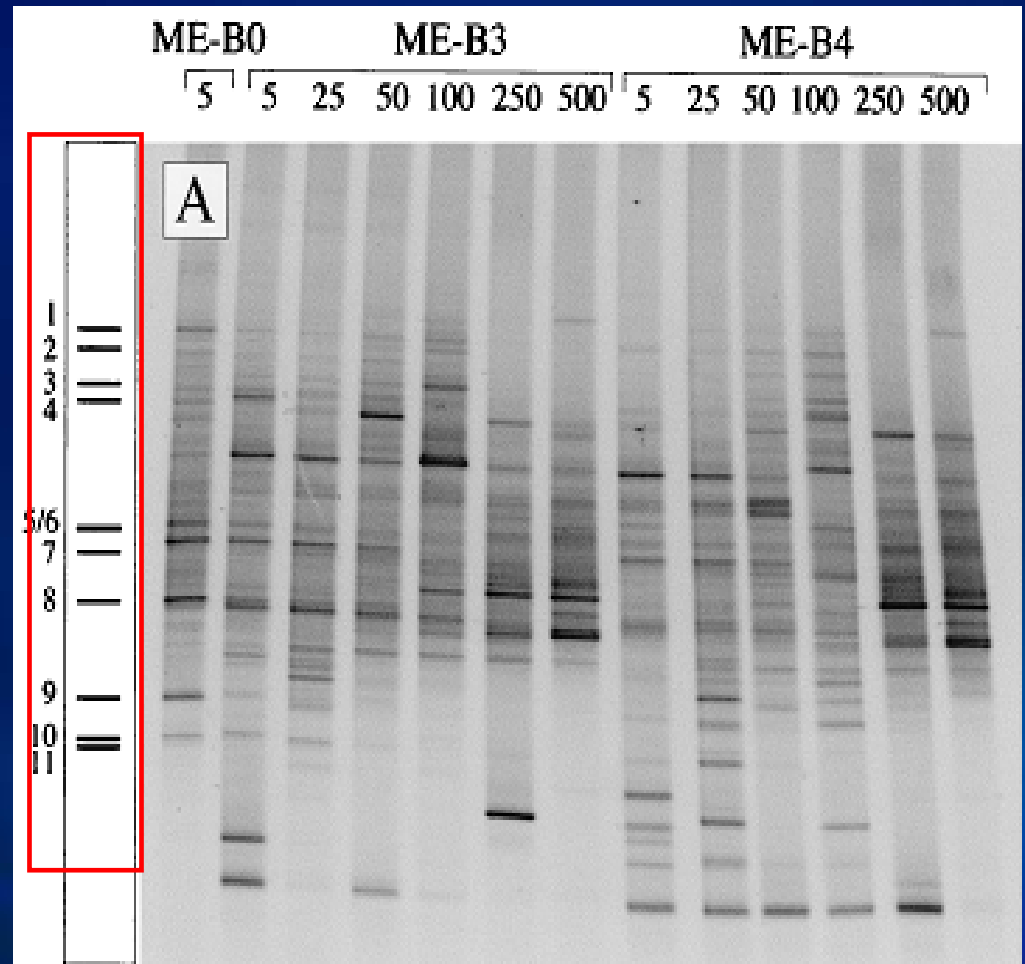
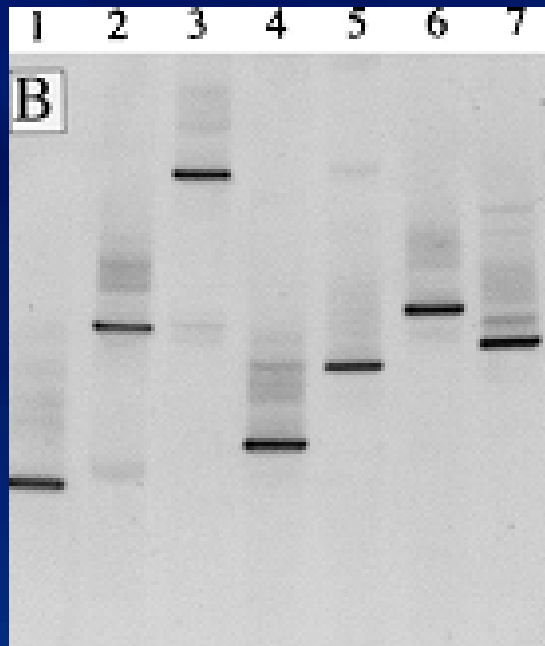


Optimization of DGGE time

Time travel experiments were performed to determine the optimal electrophoresis time. PCR products were loaded every 2 to 3 h.

After 11 h bands were clearly defined and showed reduced mobility. So the optimal electrophoresis time were 16 h .

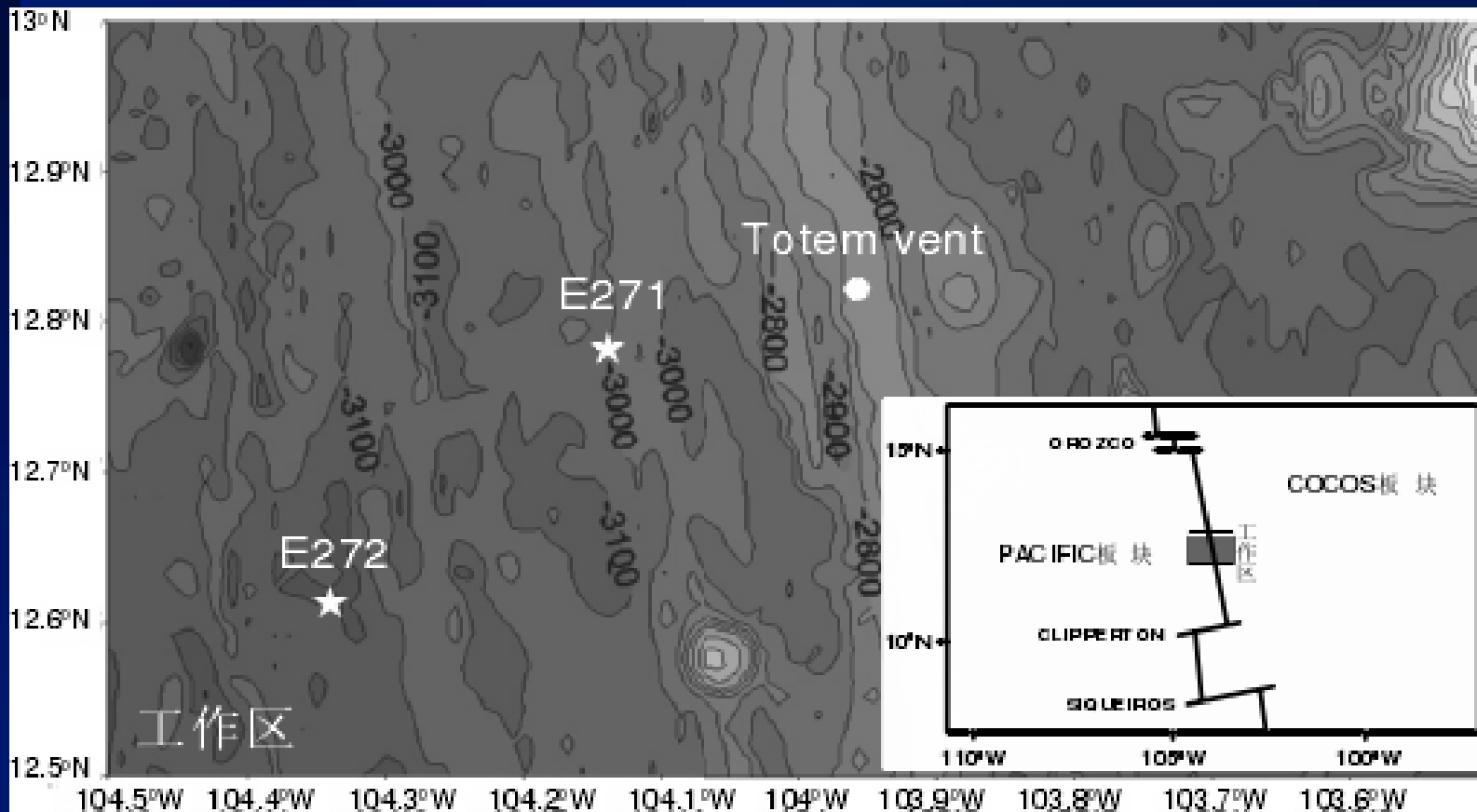




Once the optimal conditions were confirmed, DGGE was performed to analyse target samples.

Case study 2

Vertical distribution of the bacterial communities in the deep-sea sediment samples collected at the EPR



Sampling at East Pacific Rise (EPR) near 13° N: hydrothermal fields

DNA extraction

Sediment samples: every 5 cm a layer

EPRDS-1 (5-10 cm) 、 EPRDS-2 (15-20 cm)

EPRDS-3 (25-30 cm) 、 EPRDS-4 (35-40 cm)

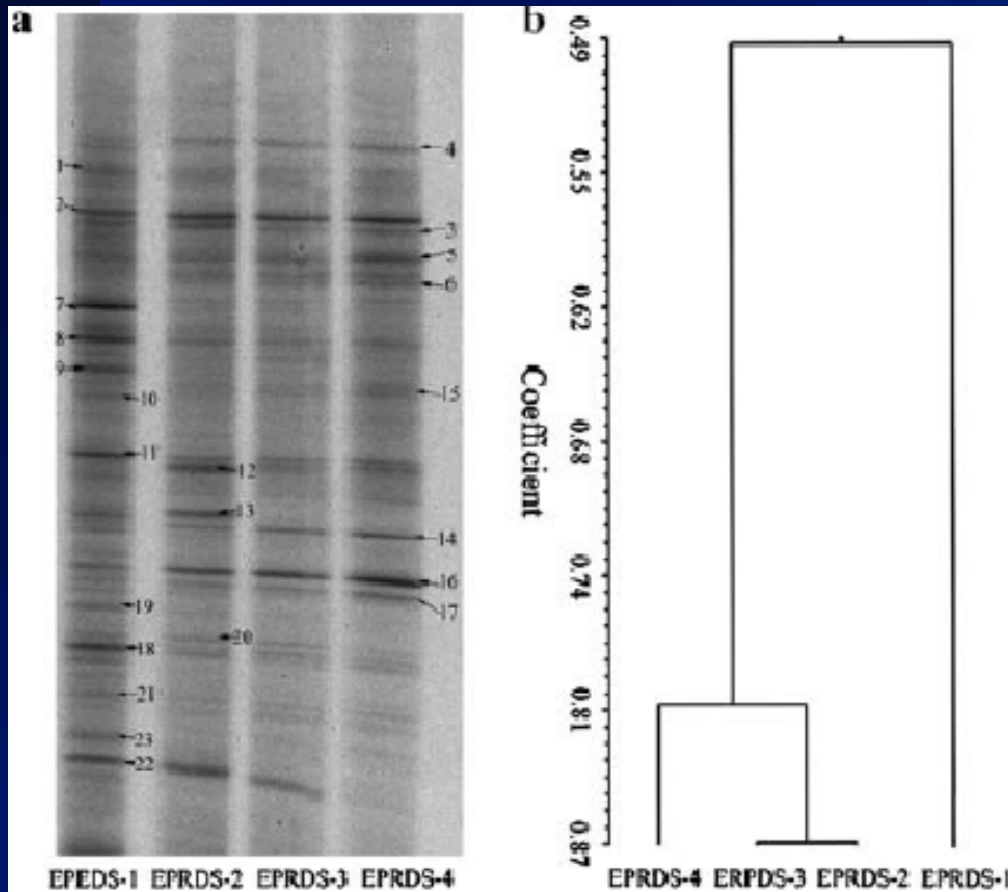
PCR amplification

16S rDNA V3 region universal primer

341F, 341F-GC and 534R



DGGE and cluster analysis



DGGE profile of bacterial communities displayed obvious difference among four sediment samples.

Results revealed that bacterial communities displayed a clearly stratified distribution along four discrete layers, which were greatly affected by the vertical zonation of geochemical features.

Phylogenetic analyses retrieved from DGGE bands

Table 2 Overview of bacterial 16S-V3 rDNA sequences detected by PCR-DGGE

| Phylogenetic affiliation | Accession number | Closest relatives | % Similarity | Environmental description | Band position |
|------------------------------|------------------|---|--------------|-----------------------------------|---------------|
| <i>Alpha proteobacteria</i> | EU259369 | Clone MD2902-B27 | 99 | Marine sediment | 7 |
| | EU259368 | Endosymbiont 1b of <i>Inanidrilus leukodermatus</i> | 99 | Marine sediment | 4 |
| | EU259366 | Clone LC1-35 | 100 | Marine sediment | 23 |
| <i>Beta proteobacteria</i> | EU259370 | Clone JH-WH208 | 99 | Soil around iron-manganese nodule | 6 |
| <i>Gamma proteobacteria</i> | EU259367 | <i>Acinetobacter</i> sp. SS-2 | 99 | | 9 |
| | EU259371 | Clone E29 | 100 | Deep-sea sediment | 2 |
| <i>Delta proteobacterium</i> | EU259358 | Clone ss1_B_02 | 96 | Marine sediment | 13 |
| <i>Actinobacteria</i> | EU259373 | Clone: NB1-i | 96 | Deep-sea sediment | 1 |
| | EU259355 | Clone AT-s2-33 | 99 | Hydrothermal sediment | 10 |
| | EU259354 | Napoli-1B-02 | 100 | Hydrothermal sediment | 11 |
| | EU259353 | Napoli-1B-02 | 99 | Hydrothermal sediment | 12 |
| | EU259356 | Clone A20 | 100 | Deep-sea sediment | 21 |
| <i>Flexibacter</i> | EU259365 | Clone SC1-26 | 95 | Marine sediment | 8 |
| | EU259364 | Clone ctg_BRRAA24 | 99 | Deep-sea environment | 18 |
| <i>Firmicutes</i> | EU259372 | Clone GASP-77KB-862-F12 | 93 | Ancient dune fields | 5 |

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|------------------------------|------------------|----------------------|--------------|----------------------------|---------------|
| <i>Planctomycetes</i> | EU259375 | clone DE6.13 | 99 | Deep-sea sediment | 17 |
| <i>Chloroflexi</i> | EU259359 | clone: ODP1251B13.5 | 98 | Hydrothermal sediment | 14 |
| | EU259360 | FS117-47B-02 | 97 | Ridge flank crustal fluids | 15 |
| | EU259362 | clone 3G02-01 | 98 | Marine sediment | 16 |
| | EU259363 | clone ref138 | 97 | Marine sediment | 20 |
| <i>Acidobacteria</i> | EU259374 | PCR-derived sequence | 97 | Hydrothermal sediment | 19 |
| <i>Unidentified bacteria</i> | EU259361 | Uncultured bacterium | 98 | Hydrothermal sediment | 22 |
| | EU259357 | Clone MD2903-B8 | 100 | Deep-sea sediment | 3 |

The high percentage of hydrothermal related members suggested the strong impact of the hydrothermalism on the surrounding environments, and sampling field was a transitional region from the hydrothermal vents to the cold sediment with great biological and ecological importance.

Case study 3

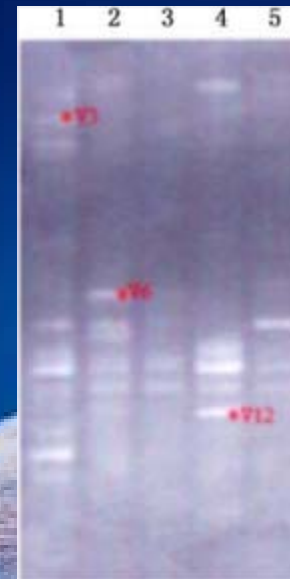
Vibrio specific primer

567F, 5'GGCGTAAAGCGCATGCAGGT-3';

680R, 5'-GAAATTCTACCCCCCTCTACAG-3'



PCR



DGGE

Development of DGGE

Double gradient denaturing gradient gel electrophoresis (DG-DGGE), a modified version of DGGE was developed by superimposing a porous gradient over the denaturant gradient.

Combined with conventional microbiology techniques, DGGE contributes to a more complete and focused description and exploration of microbial ecology.





Thank You !

**National Marine Environmental
Monitoring Center**