Marine Microbiology Diversity of China and Methodologies of Genetic Diversity Studies

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Marine Microbiology Diversity of China

INTERNATIONAL CENSUS OF MARINE MICROBES

The role of the International Census of Marine Microbes (ICoMM) is to promote an agenda and an environment that will accelerate discovery, understanding, and awareness of the global significance of marine microbes.

- **a total of approx. 5,000-10,000 thousands marine microorganisms living on our earth, but only 5,000 kinds were named.**

Culture collections of Marine Microorganisms around the world

Culture collections	Country	Adress
IMBCC	USA	University of Hawaii, 2525 Correa Road, HIG 131, Honolulu, HI 96822-2219, USA
KMM	Russia	Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, 690022 Vladivostok, Prospekt 100 Let Vladivostoku 159
MBIC	Japan	Marine Biotechnology Institute Culture Collection, 3-75-1 Heita, Kamaishi, Iwate 026-001
NCMB	UK Scotland	Torry Research Station Aberdeen, Scotland,
RCC	France	Station Biologique, Place G. Tessier, 29680 Roscoff
WCUM	U.S.A.	Center of Marine Biotechnology, 701 E. Pratt Street, Baltimore, MD, 21202

Traditional culturing:2216E

Extinction culturing:(S. A. Connon and S. J. Giovannoni,2002)

Gel microdrop: cAMP (Alke Bruns, 2002)

Different ecosystems for marine microorganisms

- **Coastal Ecosystems : Li Yun et al.,2006**
- **estuary ecosystem: Ning Xiurin et al.,2004**
- **coral reefs ecosystems: Dong Junde, et al.,2005**
- **Mangrove Ecosystems : Lin Peng et al.,2005**
- **Deep Sea Ecosystems :Mu Chunhua et al.,2005**

- Now, our database contains the information of 4627 marine microorganisms

  prokaryote :bacillus , actinomycete

  eucaryote :fungi (mould , yeast ,single-celled algae)

  Virus


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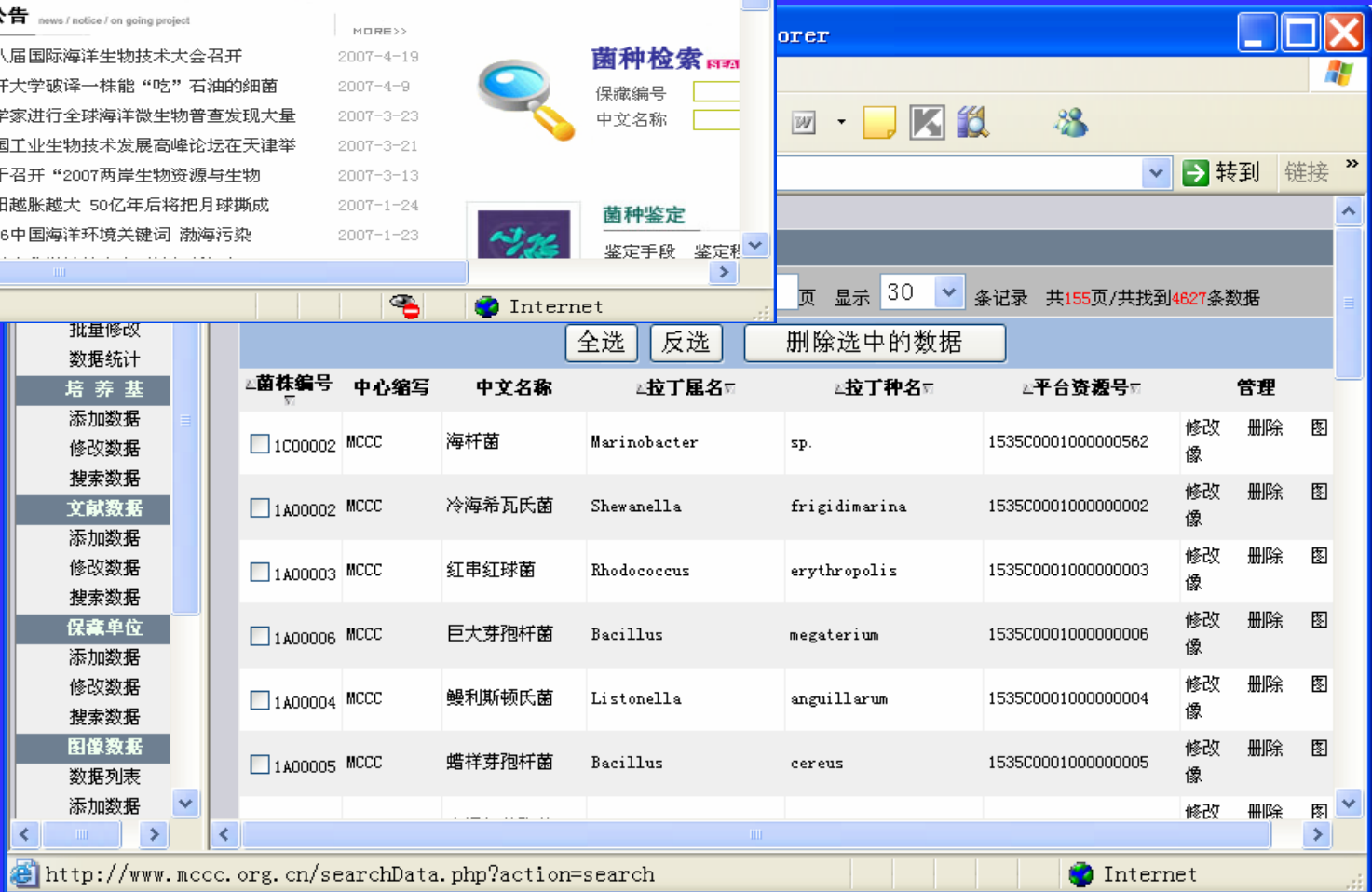
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http://www.mccc.org.cn/searchData.php?action=search
Internet

Microbial aspects of biodiversity

- It is almost impossible to quantify the abundance of an uncultured organism
- The possibility to perform environmental genomics opens a perspective to assess the potential of yet uncultured microorganisms

Methodologies of Genetic Diversity Studies

Genetic diversity

Genetic diversity is an important part of biodiversity.

In large scale, genetic diversity means the summation of every kind of genetic information carried by all the organisms on the earth.

In small scale, genetic diversity means the gene variation in the same species, which include genetic variance in the groups with significant difference of the same species and that in the same group.

Genetic diversity provides the basis to the evolution of life and differentiation of species.

1) Know the hereditary structure of natural population, which contains the genetic variance in the same natural population and between the natural populations (such as: gene frequency, the number of loci for every gene, the number of allele for every loci). Higher level of genetic diversity means higher stability of the population structure.

2) Establish gene pool of the Germplasm resounce , which is an important way to protect the Rare and Endangered organisms

3）This study is important for higher effectively cultivating : high quality, stable, yield and stress resistance .

4)Great potential will be shown with the develop of genetic diversity research about extremophiles. Because of their special physiological functions, extremophiles can survive in and adapt to some extreme environments, such as high or low temperature, extremely salty, acidic or alkaline conditions, as well as high p ressure etc. They can also, with their unique metabolic pathway formed in special environments, p roduce many special bioactive substances, such as extremozymes and antibiotics. These bioactive substances showed considerable usefulness and great potential in many areas, such as medicine, food, agriculture, chemical engineering and environmental p rotection.

5) provide scientific basis for constructive protection plan for genetic diversity through objective estimate.

Methodologies of Genetic Diversity

Protein：isozyme

PLFA:Phospholipidfattyacid

DNA：RFLP、SSR、RAPD、ARDRA、ISSR、AFLP、DGGE/TGGE、T-RFLP、DNA sequence、

ARDRA: amplified rDNA restriction analysis

ARDRA for bacteria consists of the amplification of the 16S rRNA gene, followed by separate restriction digestion with different restriction enzymes (*Hha*I, *Mbo*I and *Rsa*I *et al)*. This yields restriction patterns which in combination result in ARDRA profiles which enable differentiation between most species. And with a library of ARDRA profiles, obtained from well-identified bacterial strains ,The combination of the obtained fingerprints is designated an ARDRA profile which can be compared.

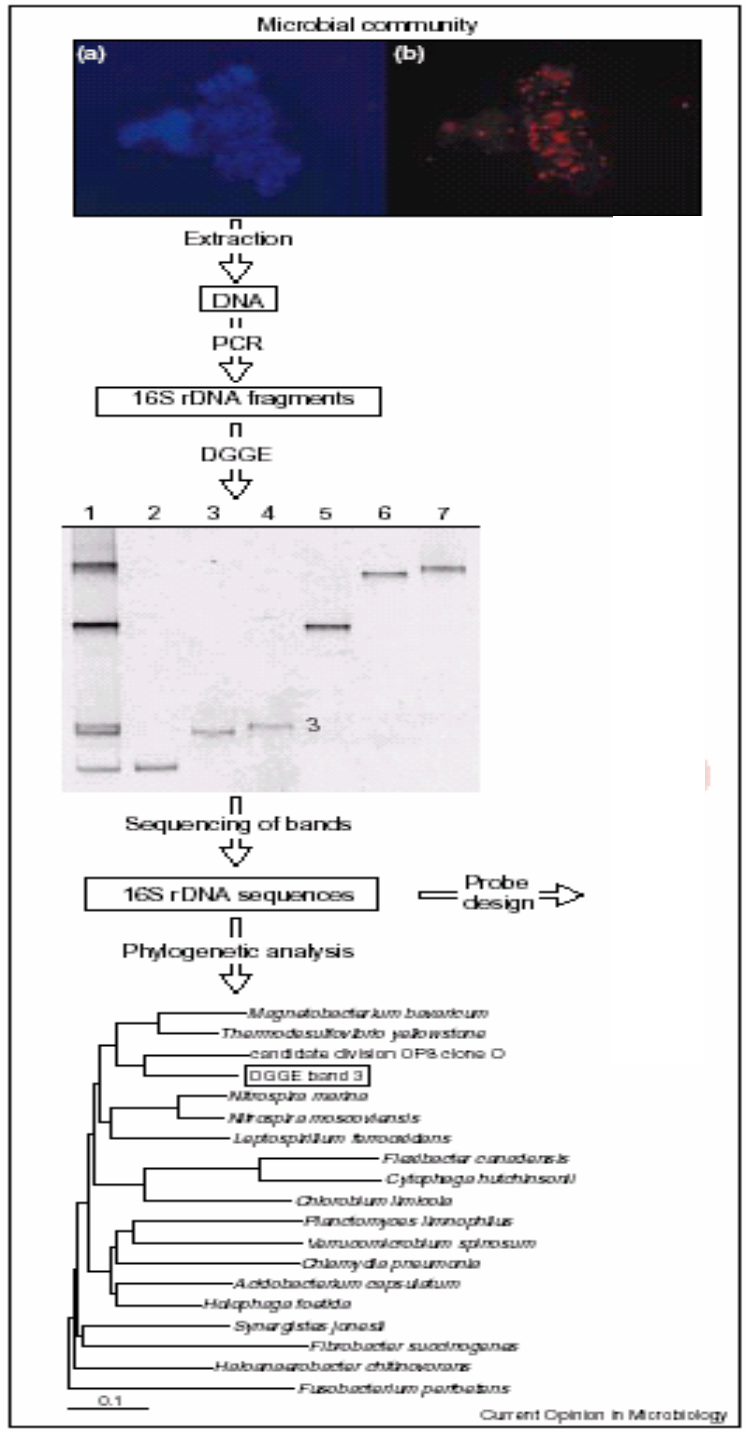
Song Zhigang,2006--A primary study on population biodiversity of Marine microorganisms from east China sea

DGGE /TGGE

Since 1994，after the introduction of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in environmental microbiology these techniques are now routinely used in many microbiological laboratories worldwide as molecular tools to compare the diversity of microbial communities and to monitor population dynamics.

Su Yunhuan,2006--Investigation on bacterial diversity of deep-sea sediments from Pacific Arctic

Tan Tianfeng,2006--Construction of M arine Oil Biodegradati0n Bacterial Consortium and Its Dynamic Change in the Process of Oil Consumption



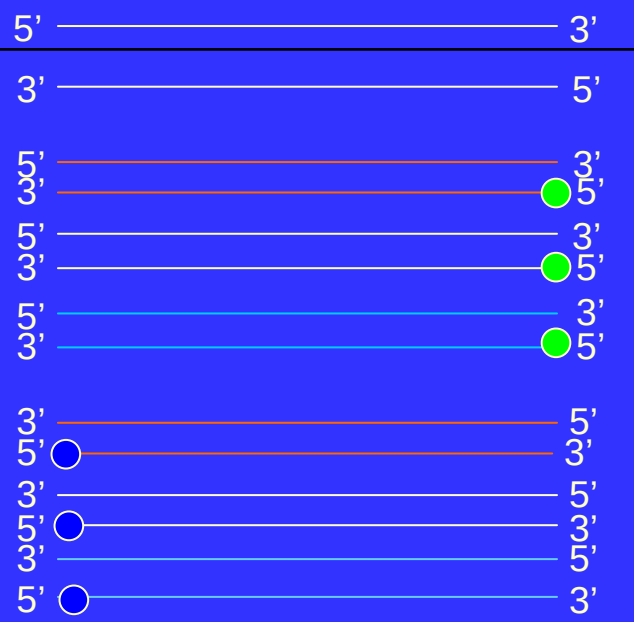
T-RFLP

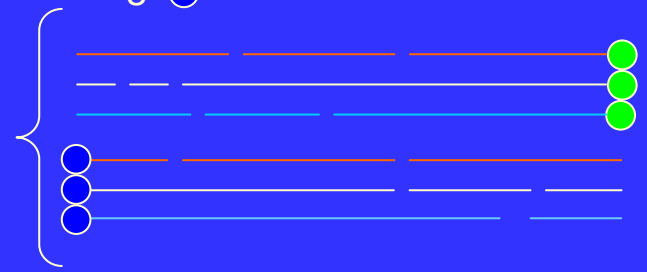
Extraction of community DNA or RNA from environmental samples

2. PCR amplification of 16S rRNA gene with fluorescent primers (HEX ,FAM or TET.)

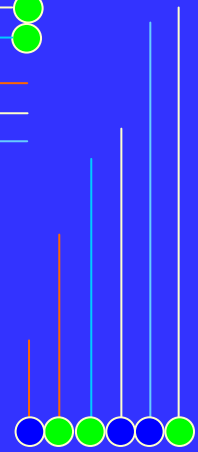
3. Digestion with one or more restriction enzymes (e.g. *Cfo*I, *Alu*I)

4. Detection and sizing of labelled terminal fragments by capillary or gel electrophoresis





*Cfo*I

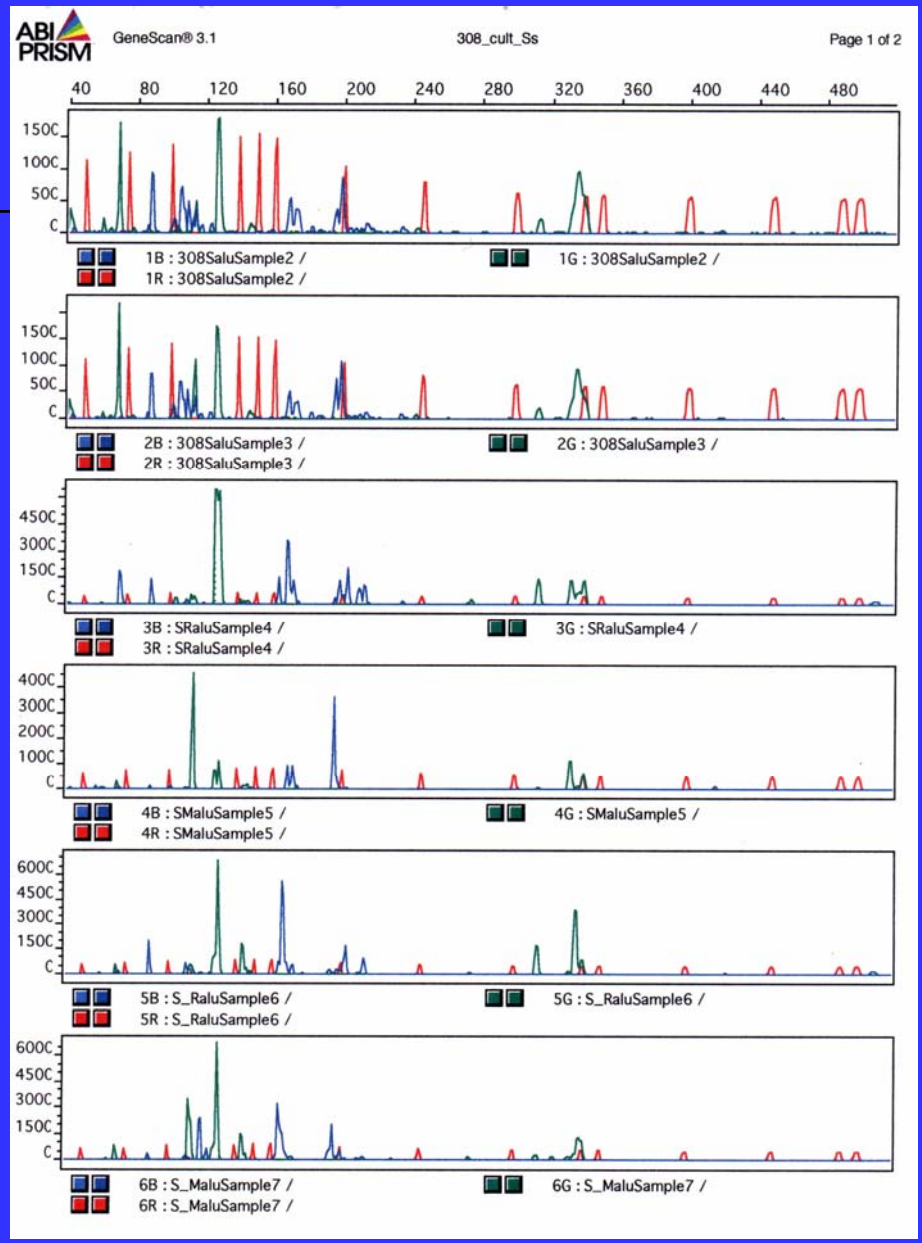


*Cfo*I profile

Cheng Haiying et al.2006---16S rRNA genes comparative analysis of microbial community in nutrient injected oil reservoir by the T—RFLP method

T-RFLP profile:Electropherogram

- Different fragments come from different organisms
- Different organisms may share a common terminal fragment size
- Peak height “in principle” correlates to the abundance of a group of organisms



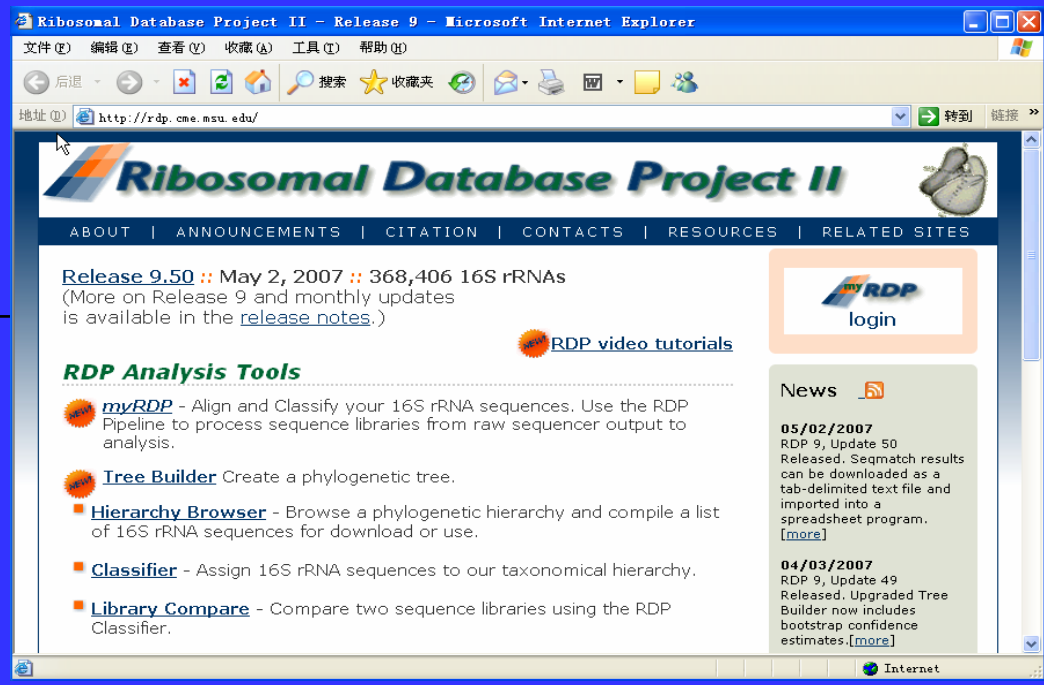
Pros and cons of T-RFLP

LIMITATIONS

- Biases inherent to PCR-based techniques
- Data generated depend on the choice of restriction enzymes
- A large group of organisms may share the same terminal fragment size
- Apparent size may differ from actual size and varies with the type of genetic analyser

ADVANTAGES

- Straightforward technique
- High reproducibility at each step of the process
- Highly precise (if not always accurate)
- Digital data can be objectively processed



DNA sequence:

- Database of rRNA:http://rdp.cme.msu.edu/
- special functional genes

Thank you