Characterization of Microbial Communities in Lakes and Reservoirs – a New Method for Cultivating Bacteria Samples from Natural Environments

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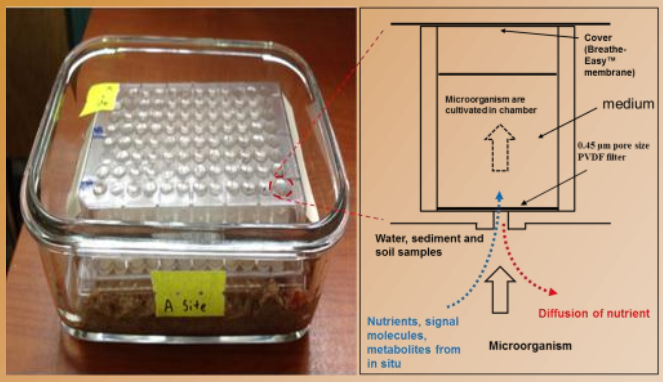
Introduction of a New Cultivation Method



Research Approach:

The majority of microorganisms from natural environments cannot be grown in the laboratory. To overcome this problem, several attempts have been made to cultivate a greater number, such as addition of signaling molecules, lowering nutrient concentrations, extending incubation time, using alternative gelling agents and in situ cultivation.

Here we developed a new in situ culture method termed "filter plate microbial trap (FPMT)" and applied it for bacterial isolation from soda and saline lakes. We used both conventional and FPMT-based cultivation to grow microorganisms from this lake, and compared the culture collections among themselves and also to a DGGE-based, culture-independent survey of microbial inhabitants.

Materials and Methods

Sampling site

Any location such as lakes, reservoirs, soils, sediments, rivers, etc. can be sampling sites. Our experiment was performed at soda lake, Buus nuur in Mongolia.

- Lake water, sediment and shoreline soil samples were collected on 11 September, 2011 from Buus nuur, Mongolia
- The water temperature, salinity and pH at the sampling site were 12.1℃, 10.9 psu and 8.9, respectively.

Filter plate microbial trap (FPMT)

1. We used UNIFILTER filtration microplates (Whatman No 7700-1806, Germany) with 96 wells as the principle growth device.
2. Each well of the plate has at the bottom a hydrophilic polyvinylidenefluoride (PVDF) membrane (0.45-μm pore-size), and sits on a plastic support with 1 mm hole.
3. The R2A media in 0.7% (wt/vol) agar was administered to each well
4. Upper part of the plate was sealed with the Breathe-Easy™ membrane to prevent contamination from the air.

Results

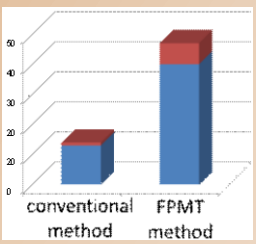


Fig 1. New species from different cultivation methods. Bars show total number of different species by conventional Petri dish and FPMT method and red bars show new species. The rate of isolation of new species was different between the conventional and FPMT-based cultivation. Only 1 novel species by conventional cultivation and 7 were obtained by using FPMTs.

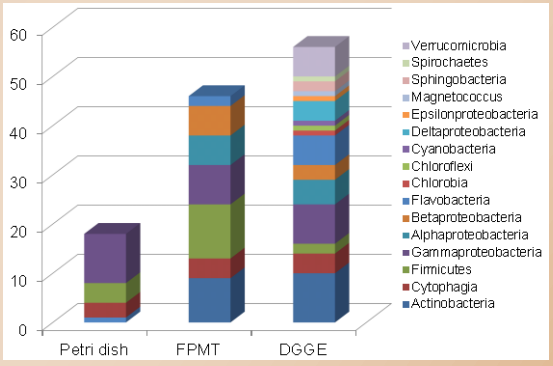


Fig 2. Bacterial taxonomic groups identified by different methods.

Table 1. Bacterial taxonomic groups obtained by different methods.

Culture-dependent		Culture-independent (DGGE)	
Conventional culture	FPMT culture	From FPMT-grown biomass	From environment sources
Actinobacteria	Actinobacteria	Actinobacteria	Actinobacteria
Cytophagia	Cytophagia	Cytophagia	Cytophagia
Firmicutes	Firmicutes	Firmicutes	Firmicutes
Gammaproteobacteria	Gammaproteobacteria	Gammaproteobacteria	Gammaproteobacteria
	Alphaproteobacteria	Alphaproteobacteria	Alphaproteobacteria
	Betaproteobacteria	Betaproteobacteria	Betaproteobacteria
	Flavobacteria	Flavobacteria	Flavobacteria
			Chlorobia
			Chloroflexi
		Cyanobacteria	Cyanobacteria
			Deltaproteobacteria
		Epsilonproteobacteria	Epsilonproteobacteria
			Magnetococcus
			Sphingobacteria
			Spirochaetes
			Verrucomicrobia
		Nitrospirae	
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The isolates from FPMT are significantly more diverse than conventional Petri dish method.

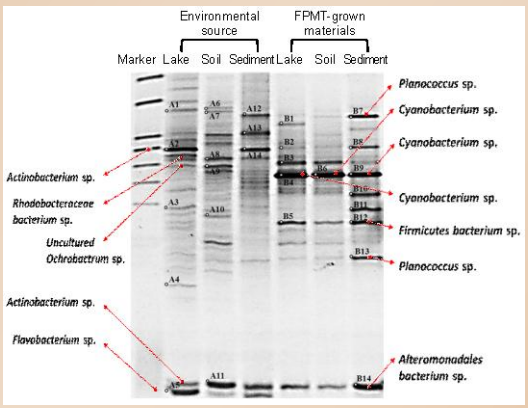

Fig 3. PCR-DGGE profiles from environmental sources and FPMT-grown materials. The number of DGGE band from environmental samples was more abundant than those from the FPMT-grown biomass.

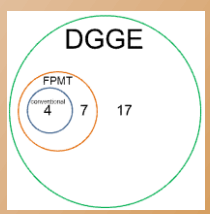

Fig 4. Overlap between identified Phyla analyzed with three different approaches (conventional Petri dish, FPMT and DGGE).

Values in the center of each circle represent the total number of Phyla from each method.

Conclusion

- FPMT-based cultivation allowed us to cultivate more microbial isolates than standard approaches
- FPMT-based culture collection was significantly more diverse and novel.
- This indicates that FPMT culture may help close the gap between the large microbial diversity in nature and much smaller diversity of currently existing culture collections.