# Domestication of uncultivated microorganisms from soil samples

Annette Bollmann[1], Lisa Ann Fagan[2], Anthony Palumbo[2], Kim Lewis[1] & Slava Epstein[1]

## Introduction

The majority of microorganisms from natural environments cannot be cultured using standard cultivation techniques. An alternative approach based on growing microorganisms in diffusion chambers incubated in the natural environment was developed (Kaeberlein et al. 2002 Science 296:1127-1129) enabling us to cultivate microorganisms directly in their habitat (Figure 1).

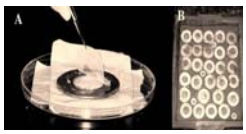


Figure 1. Diffusion growth chamber for *in situ* cultivation of environmental microorganisms.
**A**. General view.
**B.** Growth chambers incubated on the surface of marine sediment.

Preliminary data indicated that continuous cultivation of microorganisms in diffusion chambers produces variants able to grow on conventional synthetic media in the laboratory (Nichols, unpublished). Apparently the diffusion chamber produces an intermediate environment between natural conditions and growth on the Petri dish. This leads to pure cultures of previously "uncultivable" microorganisms. We refer to this phenomenon as domestication.

Using microorganisms from a subsurface soil, we evaluated the number and uniqueness of microorganisms domesticated with the chamber, as compared to microbial species obtained by standard techniques.

## Material and Methods

A soil core was taken on the FRC field site - area 3, borehole 111. The soil was diluted in sterile cold water soil extract and used to inoculate chambers and pour plates (Figure 2). The chambers were incubated for four weeks in aquaria on top of the moist soil. After the incubation the material was homogenized and used to inoculate new chambers and pour plates, also incubated for four weeks. Isolation of colonies growing in Petri dishes was done by picking all colonies with different colony morphologies visible under the dissecting microscope, and streaking them on 0.1x LB agar plates. The isolates were identified by sequencing 600-900bp of the 16SrRNA and alignment and comparison to the ARB database after preliminary Blast search.

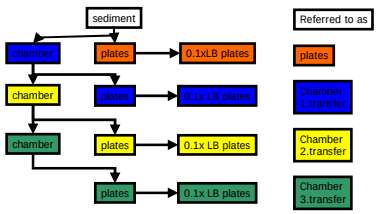


Figure 2: Experimental Set up

Table 1: Number of first time isolates from different phyla obtained by plates and chambers

| | plate | Chamber 1.transfer | Chamber 2.transfer | Chamber 3.transfer | sum |
|---|---|---|---|---|---|
| *Alpha Proteobacteria* | 4 | 15 | 2 | 10 | 31 |
| *Beta Proteobacteria* | | 3 | | 7 | 10 |
| *Gamma Proteobacteria* | 1 | 3 | | 2 | 6 |
| *Firmicutes* | | | | 1 | 1 |
| *Actinobacteria* | 8 | 4 | | 4 | 16 |
| *Verrucomicrobia* | 1 | | | | 1 |
| *CFB* | | 4 | | | 4 |

Domesticated microorganisms represented 7 bacterial phyla, whereas the readily cultivable species represented only 4 (Table 1). *Actinobacteria* were isolated equally frequently by both traditional plate- and diffusion chamber-based approaches, but almost three times more *alpha Proteobacteria* were isolated by the latter than the former. Members of the phyla *Beta-Proteobacteria*, *Gamma Proteobacteria* and CFBs could only be obtained by first cultivating them in the chambers.

Table 2: Number of first time isolates obtained by plates and chambers versus the similarity [%] with the closest cultured relative

| | Plate | Chamber 1.transfer | Chamber 2.transfer | Chamber 3.transfer | sum |
|---|---|---|---|---|---|
| 100% | 3 | 6 | | 1 | 10 |
| 99% | 4 | 13 | 2 | 14 | 33 |
| 98% | 2 | 3 | | | 5 |
| 97% | | 4 | | 3 | 7 |
| 96% | 1 | 2 | | 3 | 6 |
| 95% | | 1 | | 1 | 2 |
| 94% | | | | 1 | 1 |
| 93% | | | | | |
| 92% | | 1 | | | 1 |
| 91% | | | | | |
| 90% | | 2 | | | 2 |
| 89% | 1 | 1 | | | 2 |

Based on the alignment and calculation of the similarities, 12 out of 69 isolates had less than 97% similarity to the closest cultured relative (Table 2). Most of these isolates were isolated after domestication with the chamber approach.

In total, 58 isolates needed at least one transfer through the diffusion chamber. Some of these microorganisms grew in Petri dishes after a single transfer through the chamber, while others required up to 3 transfers before growing in Petri dishes (Table 1 and 2). The highest diversity of microorganisms was observed in Petri dishes inoculated with material after one transfer through the chambers.

Table 3: Relationship between isolates and sequences found in other experiments conducted on the FRC site. (bold indicates sequences were obtained from the contamined areas)

| Clostest relative of the isolate | Sim [%] | Fields et al 2005 FEMS Micro Ecol | Reardon et al 2004 AEM | North et al 2004 AEM |
|---|---|---|---|---|
| *R.palustris* | 99.4 | 300A-F10, 97.3% | | |
| *Methylobacterium* sp. | 99.9 | | **C-CF16**, 99.5% | **FB45-10**, 99.5% |
| *Caulobacter* sp. | 99.7 | **005C-A07**, 98.9% | | |
| *Caulobacter* sp. | 99.1 | 300E2-H11, 97% | | |
| *S. echinoides* | 99.8 | | | **FB32-20**, 97.8% |
| Bacterium Ellin 5032 | 97.5 | 300A-D04, 98.2% | | |
| *Ralstonia pickettii* | 100 | 300A-F-12, 97.3% | | **FB33-28**, 99.7% |
| *Cupriviatus necator* | 100 | **015B-D03**, 98.1% | | |
| *Imtechium assamiensis* | 98.7 | **005C-F01**, 98.1% | B-BN19, 98.5% | **FB33-14**, 99.2% |
| *P. japonensis* | 99.3 | | B-BD81, 99.6% | |
| *P. chloraphilis* | 100 | | S-H52, 98.9% | |
| *M. laevaniformans* | 99.8 | 300A-A10, 98.3% | **C-CO51**, 98.7% | |

A comparison of the obtained isolates with clone sequences from other experiments conducted at the FRC site resulted in 12 isolates with close related sequences. Seven of these clone sequences were found in contaminated areas of the FRC site.

## Conclusions

1. Transferring environmental microorganisms 1-3 times through the diffusion chamber leads to the domestication of a large number of previously uncultivated microorganisms enabling their isolation into pure culture on Petri dishes
2. Diffusion chamber based domestication selects for specific microorganisms, but these biases are different from those of traditional culture techniques
3. The diffusion chamber provides access to cultures of some microorganisms previously only known by their molecular signatures.

[1] Northeastern University, Department of Biology, Boston MA, United States, email: a.bollmann@neu.edu
[2] ORNL - Environmental Science Division, Oak Ridge TN, United States