Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

The phenomenon of microbial uncultivability

SS Epstein

Most of the microbial diversity on our planet cannot be cultivated, and remains inaccessible. To bring the missing species into culture, microbiologists have introduced over the past decade a number of innovations aiming to meet the demands of new microbes and better mimic their natural conditions. This resulted in a significant increase in microbial recovery yet the real reasons why so many microbes do not grow on artificial media remain largely unknown. The recently proposed scout model of microbial life cycle may provide a partial explanation for the phenomenon. It postulates that transition from dormancy to activity is a stochastic process originating in noise-driven bistability. The model helps explain several otherwise perplexing observations, and informs the future cultivation efforts.

Addresses
Department of Biology, Northeastern University, Boston, MA 02115, USA

Corresponding author: Epstein, SS (s.epstein@neu.edu)

Current Opinion in Microbiology 2013, **16**:636–642

This review comes from a themed issue on **Genomics**

Edited by **Barbara A Methé** and **Iñigo Lasa**

For a complete overview see the Issue and the Editorial

Available online 3rd September 2013

1369-5274/$ – see front matter, © 2013 Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.mib.2013.08.003

Introduction to the problem

The microbial richness of the biosphere is large [1], and yet its accessible, cultivable fraction is low, less than 1% [2]. This remarkable gap, 'The Great Plate Count Anomaly' [3•], was noted at the dawn of microbiology [4] and researched by the finest microbiologists of the past (e.g. [5–7]), but has not been closed. The implication is that after nearly two centuries of microbiology as a science, we know remarkably little about the overwhelming part of microbial diversity on our planet. Accessing this missing diversity is important for two key reasons: it likely plays significant roles in the function of the biosphere, and quite possibly represents an untapped mine of novel bioactive compounds [8]. Not surprisingly, learning the nature of the 'missing' diversity is widely recognized as one of the most important challenges facing microbiology [9]. This review will synthesize recent findings about the nature of 'uncultivable' microbial diversity, aiming to provide at least a partial explanation for the phenomenon.

Experiences gained

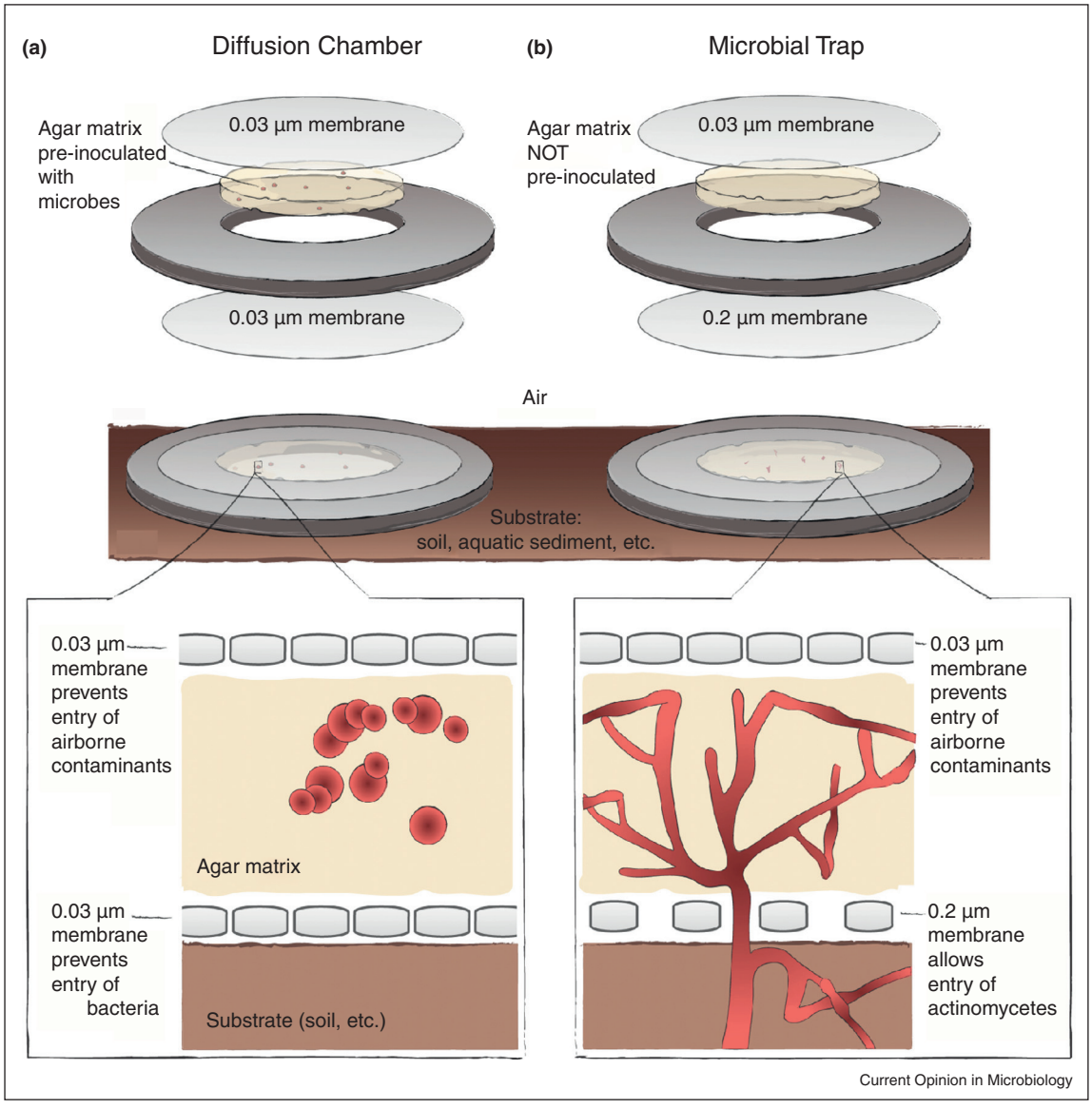
Traditionally, the cultivation microbiologist would attempt to grow novel species by manipulating the macronutrients and micronutrients in the medium, and changing cultivation conditions. While the success of this approach is undeniable, the rate of microbial discovery it affords is low: just over 7000 valid species have been described to date [10], out of perhaps millions that existed in the samples used. The last decade has seen a renaissance of novel cultivation approaches, all striving to bridge the gap.

One success story is cultivation of SAR11, a ubiquitous marine clade that until 2002 had no cultivable representatives [11•]. This study employed two ideas: dilution to extinction to minimize the influence of fast growing weeds and the use of natural seawater to facilitate growth of oligotrophs [12]. The approach was validated by successful follow up applications, and development of a higher throughput modification of the method [13,14]. A different high throughput cultivation approach based on co-incubation of cells individually encapsulated into microdroplets, under low flux nutrient conditions, also considerably increased microbial recovery [15]. Likewise, lowering nutrient concentration of standard media enabled longer incubation and resulted in isolation of species that did not appear to grow otherwise [16].

Departing from conventional thinking, Bruns *et al.* [17] explored whether addition of signaling compounds could trigger microbial growth and showed that supplementation of growth media with cAMP and homoserine lactones did increase microbial recovery. This suggests that standard media components may be necessary but not sufficient for growth of some species.

This finding is in line with the old idea that metabolites of other species may be the key to growth of many microbes. Significance of commensalisms and mutualisms in microbial world has been a popular explanation as to why many microbes refuse to grow in isolation [18], but the explicit use of co-cultivation to increase microbial recovery is a recent development. D'Onofrio *et al.* [19] directly showed that some marine microorganisms will not grow unless paired with other species, and that the critical growth factors exchanged are siderophores. How general this observation is remains to be confirmed. There are indications that, at least if co-cultivated in Petri dishes, hundreds of pairs of microbes may be required to detect partners exhibiting positive interactions (Epstein, unpubl.), especially in species from the human microbiome (K. Lewis, pers. comm).

Figure 1



The principle of *in situ* microbial cultivation. **(a)** Diffusion chamber. Environmental cells targeted for cultivation are mixed with agar, sandwiched between two 0.02-µm pore-size membranes, and returned to the natural environment of the cells for cultivation. **(b)** Microbial trap. Superficially similar to the diffusion chamber, the principle is different. The membranes have larger pores, and it is not inoculated with environmental cells, and is incubated in the environment with sterile agar inside. The pore size above 0.2 µm allows microorganisms to penetrate into the inner space, and form colonies. Both methods are based on the expectation that diffusion through membranes will establish conditions inside the device that closely mimic the natural conditions, allowing strains with unknown requirements to grow and be isolated. The trap method is more selective and enriches for filamentous microorganisms.
Reprinted from [27].

If signaling compounds and metabolites of neighbors are important growth factors, identification of the specific substances critical for growth of the given species is a challenge. One way to minimize the guess work in cultivation is to simply use the naturally occurring chemical milieu for microbial incubation. Environmental cells placed into a diffusion chamber and incubated in their own habitat will have access to the growth components from this habitat (Figure 1). Therefore, if a species grows in nature, it should grow inside the diffusion chamber, enabling cultivation of populations with unknown requirements. This idea was reduced to practice differently by several research groups, and all variations proposed to date showed significant improvement over standard cultivation [20–23,24•,25,26] (for a recent review see [27]). Of note is an interesting observation that