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Research Paper

The Great Plate Count Anomaly and the Unculturable Bacteria

Microbiology

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ABSTRACT

The term 'unculturable' is used to describe bacteria that are not grown on artificial media till date and thus these are referred as unculturables. We do not have sufficient biological information to culture these bacteria in vitro. These microscopic cells are considered to be dead and therefore would never grow posed the anomaly without classifying them. In fact, many of these cells were shown to be metabolically active, even though they were not able to grow on laboratory media.

THE GREAT PLATE COUNT ANOMALY

The great plate count anomaly is the observation that most of the microbes seen in the microscope cannot currently be grown under laboratory conditions, some may actually be nonviable, others are viable but nonculturable (VBNC). The term "the great plate count anomaly" was coined by Staley and Konopka (1985) to describe the difference between the numbers of cells from natural environments that form viable colonies on agar medium and the numbers obtained by microscopy (Fig. 1). There are several explanations for this great plate count anomaly. For example, species that would otherwise be "culturable" may fail to grow because their growth state in nature, such as dormancy, prevents adjustment to conditions found in laboratory where nutrient rich media are used for the plate counts (Deming & Baross, 2000). If an organism has a low prevalence or is particularly slow growing it is highly likely that it may have been over looked in the laboratory cultural analyses. Many genetically distinct phenotypes are phenotypically indistinguishable for example some bacteria are resistant to culture on conventional media, certain bacteria have fastidious growth requirements including the need for specific physical conditions like, pH conditions, incubation temperatures or levels of oxygen in the atmosphere (Kopke et al. 2005).

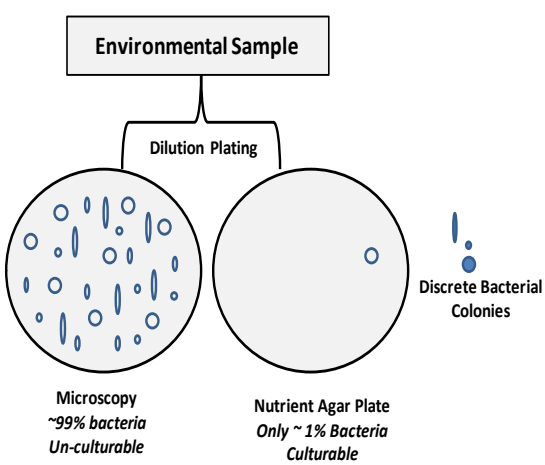

Figure 1. The Great Plate Count Anomaly

Many microorganisms are oligotrophic in nature and require fastidious conditions for successful culture. Further there may be competition for nutrients among mixtures of organisms cultured together. Growth may also be inhibited by bacteriocins released from other bacteria in a mixed culture or by antibacterial substances present within the medium (Tamaki et al. 2005). A biofilm is an aggregate of bacteria in which cells adhere to each other on a surface. This includes quorum sensing mechanism that is involved in the regulation of the bacterial community structure; properties and survival (De Kievit et al. 2001). Signaling molecules present only within the natural habitat are thought to be essential for the growth of many bacteria. In the absence of these beneficial interactions and signals, some bacteria may struggle to grow in artificial culture and may face with an unfamiliar environment devoid of essential factors (Nichols et al. 2008). Many of microbial strains that are common in nature can only be cultured by specialized techniques (Baxter & Sieburth 1984, Boogerd et al. 1989, DeBruyn et al. 1990, Koops & Moller 1992, Ferris et al. 1996, Nold et al. 1996, Partensky et al. 1996, Schut et al. 1997, Button et al. 1998, Vancanneyt et al. 2001, Wirsen et al. 2002).

THE EVIDENCE FOR THE UNCULTURED BACTERIA

The evidence for the presence of uncultured bacteria that cannot be grown in the laboratory came from molecular data. The ability to obtain DNA sequence information from an environmental sample (regardless of their viability in laboratory condition) by PCR amplification and direct sequencing allowed identification of phylogenetically important molecular markers such as 16S rRNA gene sequences (Amann et al. 1995). Such sequence information uncovered a hidden treasure of bacterial diversity that had never been acknowledged by routine cultivation. Woese described 11 bacterial phyla in 1987 which has been grown now to at least 85 divisions, majority of which have no cultured representatives (Woese 1987, Rappe & Giovannoni 2003, Keller & Zengler 2004, Achtman & Wagner 2008). The rapid appearance of members of the uncultured phyla indicates their presence in an environment. For example the TM7 phylum has been detected frequently in many different environments. A sequence corresponding to the 16S rRNA gene of TM7 was first discovered in peat bogs (Rheims et al. 1996) and it has been reported to be present in a diverse ecological conditions which include soil, water, waste treatment sludge, marine sponges and the human micro-biome (Hugenholtz et al. 2001, Hardoim et al. 2009, Bik et al. 2010, Dinis et al. 2011).

CATEGORIES OF THE UNCULTURABLE BACTERIA

The first category includes the obligatory symbiotic and parasitic bacteria. These bacteria expand under the host provided conditions but fail to grow on artificial media viz., *Prochloron didemni*, *Cristispira*, *Holospora, Caedibacter, Lyticum, Blattabacterium* and *rickettsiae*. In the second category, viable but non culturable organisms have been included. Though these organisms are viable in natural conditions but fail to undergo cell division on routinely used growth media. Cells of many native marine bacteria have been tested by a number of methods to address particularly these issues which have been observed to be alive previously but fail to grow on artificial growth media (Colwell et al. 1985).

CONCLUSION AND FUTURE PERSPECTIVE

Microbial diversity analysis of unculturable bacteria has revealed previously uncharacterized members in bacterial domains. These novel unculturable bacteria represent an unexplored and unexploited vast gene pool. Genomic library construction of unculturable members of various bacterial groups is expected to revolutionize the field of drug discovery. Availability of community genome sequences will help in

development of gene expression profiles. In addition to that an access to the neglected bacteria that reside on us may provide new avenues to improve overall health through an enhanced understanding of the yet unknown functionalities. Although advances in recent research have shown that members of bacterial groups that were previously considered to be unculturable can now be cultured, a major part of the existing bacterial diversity still remains cryptic due to their culture recalcitrance.

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