

Preface

In 1898, an Austrian microbiologist Heinrich Winterberg made a curious observation: the number of microbial cells in his samples did not match the number of colonies formed on nutrient media (Winterberg 1898). About a decade later, J. Amann quantified this mismatch, which turned out to be surprisingly large, with non-growing cells outnumbering the cultivable ones almost 150 times (Amann 1911). These papers signify some of the earliest steps towards the discovery of an important phenomenon known today as the Great Plate Count Anomaly (Staley and Konopka 1985). Note how early in the history of microbiology these steps were taken. Detecting the Anomaly almost certainly required the Plate. If so, then the period from 1881 to 1887, the years when Robert Koch and Petri introduced their key inventions (Koch 1881; Petri 1887), sets the earliest boundary for the discovery, which is remarkably close to the 1898 observations by H. Winterberg. Celebrating its 111th anniversary, the Great Plate Count Anomaly today is arguably the oldest unresolved microbiological phenomenon.

In the years to follow, the Anomaly was repeatedly confirmed by all microbiologists who cared to compare the cell count in the inoculum to the colony count in the Petri dish (*cf.*, Cholodny 1929; Butkevich 1932; Butkevich and Butkevich 1936). By mid-century, the remarkable difference between the two counts became a universally recognized phenomenon, acknowledged by several classics of the time (Waksman and Hotchkiss 1937; ZoBell 1946; Jannasch and Jones 1959).

Surely the "missing" microbial diversity was as large then as it is now. However, reading the earlier papers leaves an impression that throughout most of the 20th century the "missing" aspect was not viewed as a particularly important problem or as an exciting opportunity. A casual mention was typical of many publications. "Missing" cells were not necessarily considered missing species let alone signs of novel classes of microbes. Besides, the unexplored microbial biodiversity was a purely academic issue; the hunt for novel species as a resource for biotechnology had not yet begun. It is also important that the reasons for the Anomaly appeared rather simple at the time. Counting errors, dead cells, and later damaged cells were continuously considered significant components of the disparity. Also, it had been obvious at least since Koch's time that no single nutrient medium could possibly satisfy all microorganisms (Koch 1881), and so the finger was always pointing to media deficiencies. Indeed, imperfections in media design was such a simple and

intuitive explanation for the refusal of the microbial majority to grow *in vitro* that many microbiologists began viewing it as sufficient. The triviality of the explanation generated a perception of the Anomaly as a purely technical issue that could be resolved by bettering the media compositions and incubation conditions.

This view began to change towards the end of the 20th century. Cultivation efforts during the preceding decades did produce success stories; yet even as the manuals for media recipes grew into thick volumes, the overwhelming majority of microorganisms still eschewed the Petri dish. The progress in recovering missing species was rather incremental and did not change the overall picture. And, it was going to get worse.

The rRNA approach (Olsen et al. 1986) was a truly spectacular development: it provided insight into the microbial world missed by traditional cultivation. Novel microbial divisions were discovered by the dozen (Giovannoni et al. 1990; Ward et al. 1990; DeLong 1992; Fuhrman et al. 1992; Liesack and Stackebrandt 1992; Barns et al. 1994; Hugenholtz et al. 1998; Ravenschlag et al. 1999; Dojka et al. 2000). From the molecular surveys of the 1990s emerged an image of the biosphere with millions of novel microbial species waiting to be discovered (Tiedje 1994; Allsopp et al. 1995). What microbiologists had been able to cultivate and catalogue throughout the entire history of microbiological exploration (Staley et al. 1989) appeared to be an insignificant portion of the total. Successes in cultivation notwithstanding, the gap between microbial richness in nature and that of culture collections just would not close. Even today, most of the known microbial divisions have no single cultivable representative (Rappe and Giovannoni 2003; Schloss and Handelsman 2004). This gap was called "extraordinary" in 1932 just as it was called in 2000 (Butkevich 1932; Colwell 2000), as if the countless cultivation studies during these seventy years never existed. But, the realities of our age are different from the 1930s, and the Great Plate Count Anomaly is no longer "just" an academic observation. The need to close the gap is an urgent practical issue, as biotech and pharmaceutical industries appear to have exhausted what the limited number of cultivable species have to offer (Osburne et al. 2000). Today, the resolution of the phenomenon of microbial uncultivability is recognized as a top research priority for microbial biology (Young 1997; Hurst 2005). The principal challenges are to understand why uncultivated microorganisms are uncultivated, and to describe, access, and utilize their seemingly infinite diversity.

Microbiologists answered the call using two different strategies. One represents a group of clever approaches that bypass cultivation altogether. These go straight to the genes of the "missing" species to mine them for the information and products they encode, or employ isotopes and miniature electrodes to measure the activities of these species *in situ*. It is truly exciting to see how, today, cultivation-independent studies can be done at a single cell level. The other is a head-on strategy, and consists of a multitude of innovations in cultivation, principally aiming at mimicking natural conditions. The two strategies have their specific advantages and disadvantages, but few microbiologists think it is a battle of two competing products. Instead, the likely solution to the Anomaly is in a symbiosis between the two. What form and shape this symbiosis will take, it is too early to say, but the good news is

that both the authors and the readers of this book will likely witness, and witness soon, the process and the conclusion of this evolution.

Furthering this unification is the main goal of this volume. The contributions center around three themes. The first theme groups together several chapters that focus on what can be learned about the microbial world without cultivating it. John Bunge opens the volume by describing how to statistically estimate the size of microbial diversity using gene sequence data. Chapters by Mitchell Sogin and Terry Gentry et al. provide an account of the state of the art in recovering the sequence data from environmental samples. Antje Boetius et al. offer a perspective on studying microbes in nature by measuring their biogeochemical activities. Mircea Podar et al. explore how much information modern genomics tools can recover from single cells of uncultivated species. The second theme is the nature of uncultivated microorganisms, why so many species remain uncultivated, and how to domesticate them in the lab. Thomas Schmidt and Allan Konopka dissect the nature of slow growing species. Rita Colwell describes cells that are viable but nonculturable. Slava Epstein attempts to build a general model of the Great Plate Count Anomaly. The third theme builds connections between the Anomaly and practice. Vivian Miao and Julian Davies explore how metagenomics approaches could help to provide access to bioactive compounds produced by uncultivated species. Kim Lewis advocates a connection between the phenomenon of microbial uncultivability and antimicrobial tolerance of biofilms and persister cells. Ken Nealson's chapter concludes the volume by discussing the application of uncultivated cells to the search of life outside our planet, so as to outline the wide spectrum of subjects connected to the Great Plate Count Anomaly.

There is something else that this volume intends to convey. Working in the field of uncultivated microorganisms today involves both luck and privilege. Not everyone has the fortune to study a phenomenon that has endured for a century, is of unquestionable importance, and yet remains unresolved. It is fascinating to think that "our" phenomenon predates the model of the atom, the theory of the Big Bang, cracking the genetic code... It is humbling to think of the great minds who have contributed over the past century to its resolution. And it is sensational to think how enormously beneficial this resolution may be by providing unimpeded access to the missing microbial diversity, and the treasures therein. As to the luck ... the luck is in the timing, for the right of entry into the world of uncultivated microbes seems to be just round the corner.

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