Chapter 6.5

Deciphering the Microbial Dark Matter Using Metagenome-Assembled Genomes, Culturomics, and Seqcode

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6.5.1 INTRODUCTION

Microorganisms are fundamental to many aspects of life on earth, yet majority of them are waiting to be discovered and characterized (Sood et al., 2021). This mysterious group of microorganisms, referred to as microbial dark matter, poses a significant challenge to our understanding of microbial diversity and function to date. Traditional culturomics methods that rely on the isolation and cultivation of microorganisms are limited in their scope. These methods are also tedious and labor-intensive and many a time misses out on important connecting links within an ecosystem. To overcome this limitation, attempts were made to study the complete genetic material using available bioinformatics tools under a branch of science known as metagenomics (Handelsman et al., 1998).

Metagenomics started as a direct method of deciphering the microbial diversity of environmental samples from extreme conditions. The need of conducting metagenomic studies on experimental samples became necessary as there was an anomaly between the microscopic cell count, which was significantly higher than the corresponding counts of colony-forming units on media plates, a phenomenon termed "the great plate anomaly" (Connon and Giovannoni, 2002; Staley and Konopka, 1985). Culture-based studies have shown that about 1% of the microbial diversity is culturable using the existing standardized culturing protocols, indicating that a large number of microorganisms are yet to be identified and therefore were termed as dark matter (Dubourg et al., 2013; Lagier et al., 2016, 2018).

Recent advances in metagenomics and computational biology have provided new tools for studying this "microbial dark matter," including metagenome-assembled genomes (MAGs). MAGs are reconstructed genomes of individual microorganisms from metagenomic data and have been used to study the diversity and function of microbial communities (Eisenhofer et al., 2023). Although MAGs indicated the presence of microbes in a particular niche, naming them became problematic as existing guidelines are only for culturable microbes. Recently, tools like SeqCode have been developed as a nomenclature code for prokaryotes described from sequence data and to infer the functional potential of microbes from metagenomic data (Hedlund et al., 2022).

In this chapter, we explore the concept of "*microbial dark matter*" and the limitations of the traditional culturomics method. The advantages and limitations of metagenomics and MAGs as tools for understanding microbial dark matter have been discussed. Further, the role of SeqCode in providing nomenclature to uncultivable microbes based on genome sequence has also been covered. We end the chapter with a discussion on the current challenges and future directions in microbial dark matter research.

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