Chapter 8

Recovery and Enumeration of Viable Bacteria

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One of the greatest challenges facing soil microbiologists for more than a century has been the recovery and enumeration of “total” bacterial populations in soils. The difficulties lie not in the actual methodologies (the procedures are in fact relatively simple), but in the interpretation of results that is confounded by numerous errors arising chiefly from the nature of the substratum—the soil and the microbes themselves. It is safe to say that the dilution plate counting technique for the enumeration of microorganisms is one of the oldest and most widely used techniques in microbiology. The development of the procedures dates back to the period of Robert Koch and contemporaries (about 1880) when these pioneering microbiologists were faced with the problem of developing techniques for the cultivation of bacteria on “solid” culture media. Their efforts were directed mainly at characterizing microorganisms from a wide variety of habitats at a period when the field of microbiology (bacteriology in particular) was in its infancy. One of the earliest and lasting breakthroughs in the study of bacteria was the discovery of agar (a polysaccharide derived from marine algae) as a jelling agent for culture media. A unique and microbiologically useful property of agar is that it melts at 100 °C but does not gel until cooled to about 40 °C making it suitable for incorporating live organisms in the gel. It is also resistant to decomposition by most terrestrial microorganisms making it well suited for use as a jelling agent for studies of soil microorganisms.

Shortly after Koch’s demonstrations of the plate culture technique (which actually employed flat glass plates) Petri (1887; see translation in Brock, 1961) described the use of dishes with loose-fitting overlapping lids (the well-known petri plate or dish prevalent in all microbiology labs) for the long-term growth of microorganisms with minimal contamination from external sources. Thus, with these pioneering innovations, the foundation for the cultivation and enumeration of microorganisms by the so-called dilution plate method was laid.