33 Methods for the Study of Strictly Anaerobic Microorganisms

Kevin R Sowers[1] and Joy EM Watts[2]

[1] Center ofMarine Biotechnology,University ofMaryland Biotechnology Institute, Baltimore,MD 21202, USA; [2] Department of Biological Sciences,Towson University,Towson,MD 21252,USA

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INTRODUCTION

Most anaerobic extremophiles are obligate anaerobes that require strictly anoxic environment, and an extracellular milieu that is poised at a low redox potential. Despite their requirement for fastidiously anoxic conditions, these anaerobic microorganisms are ubiquitous in the environment and occur in a large range of ‘‘extreme’’ habitats that include anoxic sewage digestors, mammalian, ruminant and termite digestive tracts, polar lakes and tundra, geothermal submarine vents, calderas and hot springs, deep sea sediments, and deep subsurface rock. The combined processes of aerobic and anaerobic degradation ensures that CO_2 ‘‘fixed’’ as cell carbon by photosynthetic organisms and consumed by heterotrophs, is eventually restored to the atmosphere as CO_2, thus completing the global carbon cycle in even the most antianthropomorphic environments. Geological hydrogen-utilizing, CO_2-reducing extremophiles such as the methanogenic Archaea can also serve as primary producers of reduced carbon compounds in the absence of photosynthesis, which has significant implications for research in astrobiology and the search for extraterrestrial life. Unlike facultative anaerobes and oxygen-tolerant anaerobes, many obligate anaerobes require stringent anaerobic conditions to maintain viability. This is achieved by using anaerobic gases to prepare anoxic medium with the addition of chemical reducing agents to achieve a low redox potential. Specialized glassware and vessels are utilized to maintain reduced anoxic conditions during growth. This chapter describes the apparatus and methodology for growth, isolation, scale-up of the most stringent anaerobes and the isolation of oxygen-labile biomolecules. Methods described herein are applicable to methanogenic Euryarchaeota, non-methanogenic hyperthermophiles within both the Euryarchaeota and Crenarchaeota, and hyperthemophilic eubacteria within the Thermotogales. These methods are also applicable to ‘‘nonextremophiles’’ such as the iron-reducing and sulfate-reducing bacteria as well as other obligate anaerobes within the eubacteria.

METHODS IN MICROBIOLOGY, VOLUME 35
0580-9517 DOI:10.1016/S0580-9517(05)35033-1