

## Long-Term Storage of Obligate Anaerobic Microorganisms in Glycerol

A. L. Bryukhanov and A. I. Netrusov

Biological Faculty, Moscow State University, Moscow, 199992 Russia

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**Abstract**—We evaluated the possibility of storing the cultures of obligate anaerobic microorganisms (clostridia, acetogenic and sulfate-reducing bacteria, and methanogenic archaea) in 25% glycerol at  $-70^{\circ}\text{C}$  for a long time (up to 3 years). This method of storage is adequate for preserving cell viability in the majority of obligate anaerobes.

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Storage of obligate anaerobic microorganisms, most of which exhibit low growth rate, involves the conventional method of periodic reinoculations into freshly prepared nutrient media and maintenance of cultures at  $4-6^{\circ}\text{C}$ , to decrease the rate of metabolism [1]. However, this method has serious drawbacks. Thus, its weak point is the possible loss of morphological, physiological, and biochemical signs by the microorganism, which results in spontaneous mutations. Studies of obligate anaerobic microorganisms are performed by the method of periodic reinoculations. Its use is associated with a laborious procedure of preparing liquid anaerobic media for reinoculations in hermetically sealed vessels with maximum protection against the entry of oxygen (the method of Hungate) [2]. Besides this, growth of various obligate anaerobes (methanogenic archaea, acetogenic bacteria, etc.) proceeds on synthetic media of complex composition.

State University); and *C. symbiosum* (DSMZ 934) from the collection of Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Brunswick, Germany). Acetogenic bacteria *C. formicoaceticum*, *Sporomusa sphaeroides*, *Acetobacterium woodii*, *A. wieringae*, and *A. poludosum* strain Z-7390 were obtained from the Laboratory of Deleted Microbial Communities (Institute of Microbiology, Russian Academy of Sciences). Sulfate-reducing bacteria *Desulfatococcus nigrificans* subsp. *salinus* strain 435 and *D. kuznetsovii* strain 17 were obtained from the Laboratory of Microbial Biogeochemistry (Institute of Microbiology, Russian Academy of Sciences). Specimens of the genus *Thermohydrogenium*, *T. kirishiense* strain 360 and *T. lactoethylicum* strain 149, were obtained from the collection of the Department of Microbiology (Moscow State University). Methanogenic archaea *Methanobrevibacter arboriphilus* strain DH1 (DSMZ