CHAPTER 8: Maintenance and Preservation of Cyanobacterial Cultures

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Maintenance and preservation of Cyanobacterial Culture
8.1 Introduction

Survival at low temperatures is a widespread phenomenon in nature and can be observed across a wide range of organisms including cyanobacteria (Fuller, Lane and Benson, 2004; Fox, 2006; Thomas, 2005). Understanding survival mechanisms in nature and in man-made “cryobanks” is important and will assist in the sustainable exploitation of biological resources for research and biotechnology.

The maintenance of cyanobacterial cultures usually require propagating working cultures by subcultures and keeping alive and growing in the course of investigations. For a long-term preservation, cultures are usually stored in a lyophilized or deep-frozen (cryo-preservation) form to prolong their viability and to reduce changes due to the occurrence of spontaneous mutations. But, the ability of cyanobacteria to remain viable under lyophilized or cryopreserved condition can vary and can not be taken for granted. Compared to continuous sub-culturing in liquid media, growing them in slants can prove useful for all strains and mutation although can not be avoided, can be minimized.

Unlike eukaryotic microalgae (Holm-Hansen, 1973; Day and Brand, 2005), cyanobacteria can be successfully stored for long periods at −80°C, thus increasing the applicability of the approach to labs where ultra low-temperature storage facilities are not available.

8.2 MATERIAL & METHOD

8.2.1 Maintenance of working cultures

Requirements:

Agar slants in growth medium.

Procedure:

Usually, maintenance of working cultures requires the periodic transfer of strains to fresh minimal media. The cultures should be maintained in very low light (500-700 lux). They can also be streaked on cotton-plugged agar slants and allowed to grow. They can then be stored in the refrigerator in dark at 5-8°C to
reduce metabolic activity while maintaining viability; some strains can not be stored in refrigerator as they would lose all properties. Frequency of sub culturing must be kept to a minimum, but it has to be separately determined for each organism. Drying of agar must be prevented.

8.2.2 Lyophilization

Requirements:
Sterile tubes of 6mm dia, liquid N\textsubscript{2}, vacuum drying facilities & glass sealing device.

Procedure:
Small volume of samples (0.1ml) of thick suspensions of cyanobacteria in nutrient solution was pipetted into small sterile tubes (6mm outer dia.). The tubes are stoppered with a fairly loose cotton plug. The suspensions are then frozen by placing in a subzero refrigerator maintained at -25°C or by using liquid nitrogen. The frozen samples are then immediately subjected to vacuum drying in a lyophilizer unit. Once the tubes are dry, the tubes are then sealed under vacuum with a gas-air cross-fire torch and stored in dark at 5-8°C.

8.2.3 Cryopreservation

Requirements:
Cryovials and 5% methanol or 8% DMSO

Procedure:
Transfer the cultures to cryovials and pellet them. To the pellet, add 1ml of half-strength growth medium containing 5% methanol or 8% DMSO (Dimethyl sulfoximide). Shake the vials gently and avoid exposure to light, place the culture vials in a refrigerator to bring down the temperature. Then, place the vials at -70°C for 2 hours and then quickly place into a storage container and plunge into liquid nitrogen.

Cultures to be revived are removed from liquid nitrogen storage and warmed rapidly to room temperature by plunging into a dish of water at 35°C. Cells are immediately pelleted by centrifugation to remove the supernatant. One ml of
fresh medium is added to suspend the cells and vials are kept slightly loosened with dark incubation for 1 or 2 days. They can then be transferred to normal growing conditions.

8.3 REFERENCES


