

A simple strategy to purify Cyanobacterial cultures

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Abstract

The isolation of pure cyanobacterial culture from heterotrophic bacteria, fungi and actinomycetes contaminated culture is a difficult task. In order to overcome the above task, a new strategy has been developed employing the double distilled and tap water as a medium having the pH of 7.12 and 8.65 respectively. Compared to double distilled water, tap water had less efficiency in this process. Through this process, the cyanobacteria culture contaminated with bacterial contaminants is obtained as a pure culture.

Key Words: Cyanobacteria, bacterial contamination, pH, double distilled water

Introduction

The isolation of Blue Green Algae (BGA) from other contaminants like bacteria, fungi and actinomycetes has been a difficult task. The growth rate of the cyanobacteria is very low when compared to the growth of contaminating bacteria and fungi which results in the depletion of nutrients from the medium. The carbohydrates synthesised by BAG such as glucose, mannose, xylose and ribose (Parikh and Madamwar, 2006) can be used as a carbon source for the contaminants. The bacterial contaminations have been continuously checked by plating on Luria broth (LB) and incubated at 37° C (Henson *et al.*, 2004).

Due to the presence of contaminations, the estimation studies of chlorophyll, genomic DNA and an extra cellular study of polysaccharides and protein profile have been affected. In case, if the contaminated BGA cultures which have a resistant to lysozyme, the pure genomic DNA of those BGA can be isolated employing lysozyme along with Dnase. The use of Lysozymes and Dnase was not cost-effective. Various approaches (Allen, 1952; Ferris and Hirsch, 1991) have been tried to develop more-efficient methods to purify contaminated cyanobacteria. This includes mechanical separation of the cyanobacteria and bacterial contaminants by

micromanipulation, differential filtration and repeated transfer of cells (Allen, 1952). Other approach involves the use of an agent which was judged to be relatively harmless to the cyanobacteria but toxic to the bacterial contaminants. The various process parameters which are used for pure culture are phenol, sodium hypochlorite, detergents, sodium sulfide, UV or gamma irradiation, elevated temperature, and antibiotics (Ferris and Hirsch, 1991).

Among all the above process, the use of antibiotics to obtain pure culture is the most common one. Even for some experiments, BG-12 and BG-13 media have been supplemented with cycloheximide and nystatin, each at a concentration of 100, g ml⁻¹ (Ferris and Hirsch, 1991) and also imipenem which will inhibit the bacterial peptidoglycan biosynthesis (Kropp *et al.*, 1985). In addition to the above, it is suggested that to use fresh and filter sterilized aqueous stock solutions (0.5%) of cefoxitin, ampicillin and penicillin G. The BGA germplasm collection centers are largely depending on antibiotics to get the pure culture, which is very expensive. The draw back of using antibiotics in the culture medium needs the resistance by the BGA to the antibiotics.

Most of the bacteria, fungi and actinomycetes that contaminate BGA culture were heterotypic in nature which depends on the

nutrient present in their environments. However, BGA's are autotrophic in nature. Based on this principle, the BGA culture has been purified from their contaminants.

The BG11 media used for cyanobacterial cultivation is the nutrient source of contaminating heterotropes. For BGA cultivation there is no need of using all components of BG11 media. Instead of BG11 media, sufficient base for their growth can be provided by both tap water and distilled water as a medium. Due to 0% nutrient supply in the above media, the heterotropes growth can be arrested.

Materials and Methods

In this process, 100 ml of tap water and double distilled water were checked for pH before and after sterilization separately. From the bacterial contaminated BGA culture, 0.5 ml was transferred to water medium as an inoculum. The culture was incubated at 25 ± 2° C with light intensity of 3 to 5 k lux in 11/13 h light and dark cycle. The 5 days old water BGA culture was taken and checked under microscope for contamination. One can overcome this problem, the aqueous culture of BGA was washed in sterilized double distilled water to remove the contaminating cells or spores adsorbed on the algal mass and sub-cultured in another sterilized aqueous medium. The continuous sub-culturing was carried out in the same medium which helps to obtain effective results. The purity of the culture was checked under light microscope. This process is very much helpful to reduce the cost to obtaining pure BGA from contaminated cultures.

Result and Discussion

The cyanobacterial culture (*Westulopsis* spp.) contaminated with heterotrophic

Table-1 Measurements of physical parameters of the water medium

	Double Distilled Water		Tap Water	
	Before sterilization	After sterilization	Before sterilization	After sterilization
pH	7.1	7.12	8.26	8.65
Dissolved Oxygen mg L ⁻¹	6.68	7.24	6.52	6.50

bacteria (Fig.1 a & b) was purified employing water medium (Fig.1 c & d). The pH values of non-sterilized and sterilized double distilled water and tap water are respectively, 7.1 and 7.12, and 8.26 and 8.65. The pH and dissolved oxygen content of both the water mediums are given in Table 1.

The prolong incubation of BGA culture in the water medium enhances the growth of contaminants. This may due to the production of extra cellular polysaccharide in the aqueous medium by the cyanobacteria. The repeated sub culturing of BGA in water media helps to overcome this problem.

It is observed that the tap water contains mineral salts while there is no mineral salt in double distilled water. Thus, the tap water

stimulates the growth of BGA as well as contaminants leads to less efficiency. The dissolved oxygen concentration of double distilled and tap water is also measured before and after sterilization. It is evident from the above studies that the efficiency of obtaining pure culture is high for double distilled water when compared to tap water.

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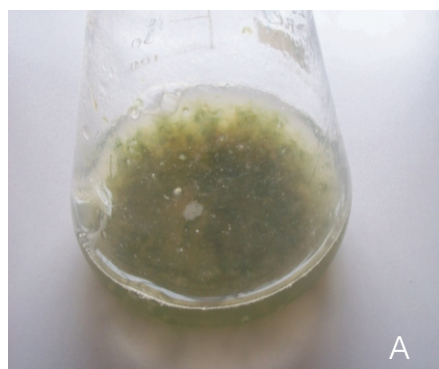
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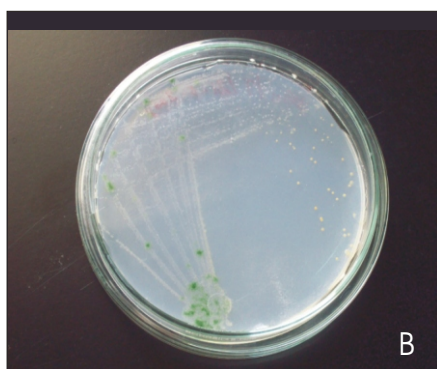
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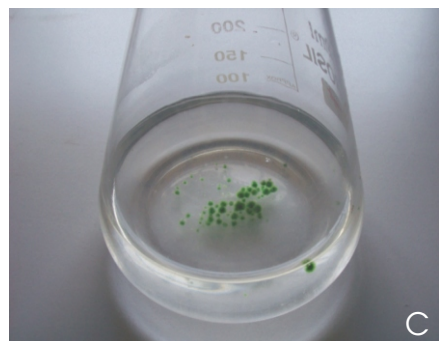
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A) BGA culture having contaminants in BG 11 suspension medium



B) BGA culture having contaminants in BG 11 agar medium



c) & d) Purified BGA culture in water medium

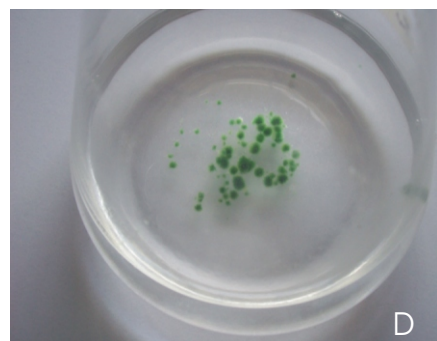


Fig 1. The *Westulopsis* spp. (cyanobacteria) cultures before and after process