ESTIMATION OF PROTEIN AND DETERMINATION OF PROTEIN SUBUNITS OF *CHLOROCOCCUM INFUSIONUM* (SCHRANCK) MENGHINI

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Abstract: The common unicellular green algae *Chlorococcum infusionum* can be used as a source of protein like *Chlorella*. Hence it contains 42% of dry weight protein. The present findings showed that when the culture media is formulated with urea (a nitrogenous fertilizer), the protein content observed was maximum than the other medium formulations. SDS-PAGE electrophoresis showed 5 protein bands of molecular weights 42, 29, 26, 16, 14.3 k D. So it may be used as a supplement for protein in future.

Keywords: *Chlorococcum infusionum*, Media formulation, Protein subunits, SDS-Page

INTRODUCTION

Microalgae composed of carbohydrate, protein, lipid and minerals which are used as nutritional supplement for human consumption (Richmond, 1989; Brown *et al*., 1989). Nearly 30 to 60% of protein is observed from microalgae (Watanabe, 1993). Today more than 40 different species of microalgae are isolated and cultured as pure strains in different parts of the world (De Pauw and Persoone, 1988). They belonged to 8 different classes. Japan was the first country to culture *Chlorella* biomass as health food (Iwamata, 2004). The term single cell protein (SCP) refers to dead, dry cells of microorganisms such as yeast, bacteria, fungi and algae. The dried cells of microorganisms or the whole organism is harvested and consumed as a protein source. The potential use of Chlorophyta stands in a higher level and different culture media are chosen by the algologist for culture studies. The objective of the present study is to find out the percentage of protein content in different media formulations and to determine the protein subunits of the unicellular green algae *Chlorococcum infusionum*.

MATERIALS AND METHODS

For the present study the pure culture of *Chlorococcum infusionum* was isolated by serial dilution method and grown in Allen's medium (1968). The growth characteristic of the selected green algae was assessed by culturing them in different formulated media, having different nutrient sources under laboratory conditions. In the experimental culture media, the pH was maintained at 6.9 and kept in a constant temperature of 28 ± 1ºC with 12 hours light/dark condition. The illumination was made by fluorescent lamps (2000 lux). All the culture flasks are kept in a shaker for constant moving and are kept in triplicates with control.

Media formulation

Media formation 1 (mf_1)

Using urea as a nitrogen source, MF_1 media was prepared with varying concentrations from 0.1% to 1% by dissolving appropriate amount of urea in one litre Allen's medium.

Media formulation 2 (mf_2)

Using potash as a nutrient source, MF_2 media was prepared with varying concentrations from 0.1% to 1% by dissolving appropriate amount of potash in one litre Allen's medium.

Media formulation 3 (mf_3)

Using *Azospirillum* as a source of biofertilizer, MF_3 media was prepared with varying concentrations from 0.1% to 1% by dissolving appropriate amount of *Azospirillum* in one litre Allen's medium.

The media of all the above mentioned concentrations were kept in sterilized separate conical flasks and labelled.

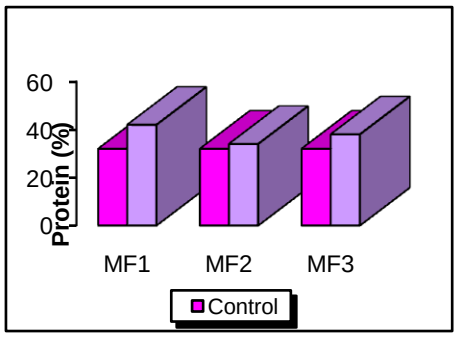
Inoculation of the medium

20 cells/ml stock culture of *Chlorococcum infusionum* was introduced into each set of media formulations (MF_1, MF_2, MF_3). The cells are harvested during the stationary phase for the estimation of protein.

Estimation of protein and determination of protein subunits

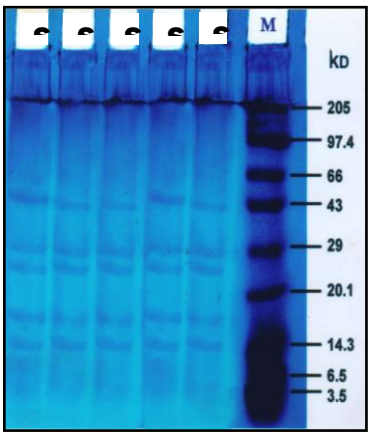
The protein content of *C. infusionum* was estimated by Lowry *et al*. (1951). Using SDS-PAGE electrophoretic method (Laemmli, 1970) the molecular weights of protein subunits were determined.

Fig. 1. Protein content of *Chlorococcum infusionum* cultured in different media formulations.



MF_1-Urea,MF_2-Potash,MF_3-Azospirillum

Fig. 2. Molecular weight determination of *Chlorococcum infusionum* protein.



RESULT

MF_1 showed the maximum percentage of dry weight of protein (42%). MF_2 and MF_3 showed 38% and 34% of dry weight of protein respectively (Fig. 1). The control shows 32% of dry weight of protein. There were five protein bands determined by SDS-PAGE electrophoresis. The molecular weight of the protein subunits were 43, 29, 26, 16 and 14.3 k Daltons (Fig. 2).

DISCUSSION

In the field of aquaculture, microalgae like *Chlorella*, *Spirulina Scenedesmus* and *Dunaliella* were used as food for their high protein content (SCP) (Brown *et al*., 1989). Kumar (1990) had observed the success of culturing *Spirulina*, the major source of protein in India. It has been demonstrated that different nutrient sources alter the constituent of protein, carbohydrate, amino acid and lipid of microalgae (Utling, 1985; Sunneik and Wabnon, 1991). *C. infusionum* grown in MF_1 (Urea) showed 11% more protein than the control. In the present investigation, nitrogenous fertilizer accounted for maximum protein concentration. MF_1 medium (Urea) showed maximum percentage (42%) of protein (Fig. 2). In the culture studies of *Chlamydomonas reinhardii*, *Chlorella vulgaris* and *Dunaliella salina* the protein content was 48%, 58% and 57% respectively (Pauline et al., 2006). Five protein bands (Fig. 2) were determined by SDS-PAGE electrophoresis with molecular weights of 43, 29, 26, 16 and 14.3 k Daltons. *Chlorococcum infusionum*, the common unicellular green algae can be used as a source of protein to improve the food value of aquaculture feed. It may be an alternate for the problem of protein scarcity.

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