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Isolation of New Isolate of Micro Algae *Chlorella* sp. *Al*-25 from Tiab Estuary of Iran

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Abstract: A saline water micro algae was isolated from Tiab estuary. The Al-25 strain was identified to be as genus *Chlorella*. The maximum number of viable cells was $50/8\times10^4$ CFU mL^{-1} with 852 mg L^{-1} of DCW and the maximum specific growth rate and biomass productivity were estimated to be 72 h and 852 mg L^{-1}, respectively. Crude protein content of *Chlorella* Al-25 was about 55-58%.

Key words: Algae, *Chlorophyta, Chlorella*, identification keys, total protein, Kjeldahl method

INTRODUCTION

The green algae of the genus *Chlorella* (Beijerinck, 1890) belongs to the family of *Chlorellaceae* (Hoek *et al*., 1995). Species of this genus are widespread in fresh water and in the sea, air and soil. In asexual reproduction stage this micro algae are produce none motile autospore. It is becoming increasingly evident that the development of low-cost, high-quality protein feed is crucial for the future success of aquaculture industry (Rumsey, 1981). A Single Cell Protein (SCP), microalgae have been used as essential food for the larval stages of fish and shellfish (Benemann, 1992) and yeast and bacteria have been considered as algal substitute for several species of filter feeders (Epifanio, 1979a, b; Kim *et al*., 1998). *Chlorella* are also used for microbial protein production and as protein rich food for sewage oxidation (Kessler, 1982). The *Chlorella vulgaris* has ability to produce carotenoid of lutein which is very important in human serum and food as well (Li *et al*., 2001). *Chlorella* species are very simple unicellular algae and also the members of this genus are easy to cultivate and wildly used in various physiological studies. The identification of *Chlorella* species is difficult because morphological and physiological characteristics normally changed with the environmental conditions. However the *Chlorella* cells do not exhibit characteristics that differentiate them from the morphological properties which are typically the basis of the classical taxonomy treatment of other algae (Shihira and Krauss, 1965). Although the traditional taxonomic characteristics of *Chlorella* sp. indicate that morphological, biochemical and physiological properties are used in its identification, the cells size and shape are variable and largely depend on varying nutrition and environmental factors (Fott and Novakova, 1969). The objective of this study was to isolate, identify and purify *Chlorella* sp. from Tiab estuary in Iran.

MATERIALS AND METHODS

This study was carried out at the University of Alzahra and A.C.E.C.R., Water sampling was done from spring of 2004 to winter of 2005. Water samples as an isolating source of micro algae were collected from Hormozgan province, Tiab estuary in Persian golf of Iran. Micro algae were newly isolated from water samples by the methods described by Watanabe *et al*. (1992) and APHA/AWWA/WPCH (1989).

***Chlorella* identification:** Micro algae were identified as described by Carmelo (1997) and Kessler and Huss, (1992).

Morphological observations: Algal samples of each *Chlorella* species were observed with a zeiss microscope equipped with Normarski interference optics and the number of endospores and the size of 100 cells of each isolate were measured with a micrometer eyepiece. The observations were made at different stages of the life cycle, either on cells in exponential phase or on cells in late stationary phase of growth.

Culture conditions: Table 1 shows the details of culture conditions. Two identified the micro algae were isolated from different regions at first the ability of these strains to grow on different media has been evaluated. Table 2-5 shows the details of components of these media. The

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Table 1: Details of cultural conditions

Photo period	Light (LUX)	Temp. (°C)	Airing
12-12	3000-3300	26-28	Mild

Table 2: Specific culture media for isolation of *Chlorella strain Al-25*

A solution compound	Quantity
$NaNO_3$	3 g
$CuSO_4$	0.0004 g
$CoCl_2$	0.0008 g
$MnCl_2$	0.27 g
$FeCl_2$	0.24 g
$ZnCl_2$	0.03 g
H_3BO_3	3.44 g
	Distilled water: 900 cc
B solution	Quantity
Na2EDTA	3 g
$CuSO_4$	0.0004 g
$CoCl_2$	0.0009 g
$MnCl_2$	0.27 g
$FeCl_2$	0.24 g
$ZnCl_2$	0.3 g
H_3BO_3	3.1 g
	Distilled water:1000 cc

9 cc from A solution and 1cc from B solution add to 1 L of culture medium

Table 3: B medium

Compound	Quantity
$NaNO_3$	(75 g L^{-1} dH_2O) 1.0 mL
$NaH_2PO4.H_2O$	(5.0 g L^{-1} dH_2O) 1.0 mL
$Na_2SiO_3.9H_2O$	(30.0 g L^{-1} dH_2O) 1.0 mL
B trace Metal Solution	1.0 mL
$FeCl_3.6H_2O$	3.15 g
$Na_2EDTA.2H_2O$	4.36 g
$CuSO_4.5H_2O$	(9.8 g L^{-1} dH_2O) 1.0 mL
$Na_2MO_7O_4.2H_2O$	(6.03 g L^{-1} dH_2O) 1.0 mL
$ZnSO_4.7H_2O$	(22.0 g L^{-1} dH_2O) 1.0 mL
$COCl_2.6H_2O$	(10.0 g L^{-1} dH_2O) 1.0 mL
$MnCl_2.4H_2O$	(180.0 g L^{-1} dH_2O) 1.0 mL
Distilled water	1000 cc
B vitamin	
Vitamin B_{12}	(1.09/LdH_2O) 1.0 mL
Biotin	(0.1 g L^{-1} dH_2O) 10.0 mL
Thiamin HCl	200 mg
Distilled Water	1000 cc

Table 4: C medium

Compound	Quantity
KNO_3	100 g L^{-1} dH_2O
NaH_2PO_4	10 g L^{-1} dH_2O
Na_2SiO_3	1 g L^{-1} dH_2O
$FeCl_3$	3 g L^{-1} dH_2O

Note: 1 cc of every stock solution of C medium add to 1 L of sea water

micro algae culture experiments were conducted to determined the culture conditions of the isolates. The algal strains were grown in 250 mL Erlenmeyer flasks contains 25 mL of modified Sato medium (Richmond, 1983) with 3 g L^{-1} $NaNO_3$ as a nitrogen source and a pH adjusted to 8.1 by adding $NaHCO_3$. The initial pH of the medium was 7.8. Cultures were incubated for 5 days at 26 to 28°C under a 12 h light: 12 h dark period using cool white fluorescent light and also very mild aeration is provided. Table 1 shows the details of cultural conditions. For the salt tolerance tests the algae were grown at different concentrations of NaCl (2, 4, 6 and 8%).

Table 5: D medium (9), basis of mineral materials solution

Compound	Quantity
KNO_3 or $NaNO_3$	116 or 100 g
Na_2 EDTA	45 g
H_3BO_3	33.6 g
$NaH_2PO_4.4H_2O$	20 g
$FeCl_3.6H_2O$	1.3 g
$MnCl_2$	0.36 g
Micro nutrient solution	1 mL
Distilled water	1000 mL
2-micro nutrient solution	
$ZnCl_2$	2.1 g
$CoCl_2$	2 g
$(NH_4)_6MO_7O_{24}.4H_2O$	0.9 g
$CuSO_4.5H_2O$	2 g
Distilled water	100 mL
3-vitamins solution	
B_1	200 mg
B_{12}	20 mg
Distilled water	100 mL
1 mL	Basis of mineral materials solution
0.1 mL	Vitamin solution
1000 mL	Sea water filtered with 0.2 μ filter

Assay: The algal growth and Cell density was assessed by measuring the absorbance at 660nm using a Spectrophotometer (LI-250 CECIL, Inc.,UK).

Protein extraction: Total protein were exterated using a Lowry method as described by Lowry *et al.* (1951) and also micro kjeldahl method (APHA, 1985). The concentration of total protein was determined by measuring the absorbance at 660 and 730 nm using a spectrophotometer (Hellebust and Craigie, 1973).

RESULTS AND DISCUSSION

Isolation of micro algae: A new saline water micro algae was isolated which could grow well in modified Sato media. It is a single cell green algae named to *Chlorella* strain Al-25 and the cell size is about 5 microns referred to Fig. 1. The Al-25 strain was identified as genus *Chlorella* according to the Beijerinck (1890). *Chlorella* Al-25 was cultured under 12 light:12 h dark period using cool white fluorescent light (3000-3300 LUX) at 26°C to 28°C. Growth characteristics including: Total chlorophyll and maximum cell concentration measured by using Haemocytometer lam method. Present results indicated that *Chlorella* Al-25 were grown in different salt concentration medium in this study. The maximum dry weight biomass was obtained with 852 mg/L/dw of biomass after 72 h cultivation. Growth characteristics were evaluated by the following two characteristics the linear growth rate and the maximum cell concentration. Figure 2 shows the growth of the *Chlorella* Al-25 strain. Growth curve was produce for *Chlorella* strain Al-25, growing in modified Sato broth using Optical Density (OD), measurements at 660 nm. The results from the growth

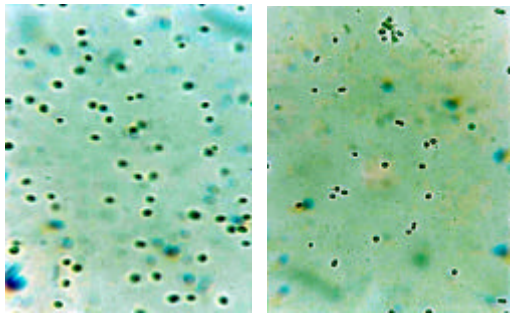

Fig. 1: *Chlorella* sp.

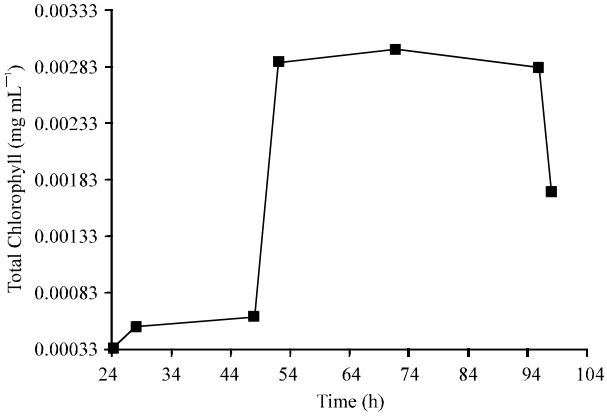

Fig. 2: *Chlorella* growth curve

curves of the *Chlorella* strain Al-25 tested in sato broth indicate that maximum of algae cells concentration was after 72 h cultivation. The growth rate was remarkably low after 48 h cultivation with maximum cells concentration with 188 mg/L/dw of biomass and reached to the stationary phase after 72 h. *Chlorella* strain Al-25 was cultured in sato medium for single cell protein production. *Chlorella* strain Al-25 cultured in sato broth under shaker-grown condition.

The total protein was extracted as described by Lowry *et al.* (1951)and micro kjeldahl. The results of total protein extraction shows that *Chlorella* strain Al-25 produce 3/15 mg mL^{-1} of medium. The crude protein content of *Chlorella* strain Al-25 was about 55-57 %. This protein content was higher than yeast cells (50/5%) (Kobayashi and Kurata, 1978), *Cellulomonas* sp. (44%) and was lower than most photosynthetic (Thanikachalam and Rangarjan, 1986). These results show that *Chlorella* Al-25 is a promising strain to cultivate for protein production.

REFERENCES

APHA., 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edn., American Public Health Association, Washington, DC., pp: 412-441.

APHA/AWWA/WPCH, 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edn., American Public Health Association, Washington, DC., pp: 225.

Beijerinck, M.W., 1890. Culturversuche mit zoochlorellen, lichenin-goinden und anderen neideren. Algen. Bot. Ztg. 45: 726-739.

Benemann, J.R., 1992. Microalgae aquaculture feeds. J. Applied Phycol., 4: 233-245.

Carmelo, R.T., 1997. Identifying Marine Phytoplankton. Academic Press, Florida, pp: 844-858.

Epifanio, C.E., 1979a. Comparison of yeast and algae diets for bivalve mollusks. Aquaculture, 16: 187-192.

Epifanio, C.E., 1979b. Growth in bivalve mollusks: Nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* and the hard clam mercenaria. Aquaculture, 18: 1-12.

Fott, B. and M. Novakova, 1969. A Monograph of the Genus *Chlorella*. In: Fott, B. (Ed.). the Freshwater Species, Studies in Phycology, Academia, Praha, pp: 10-74.

Hellebust, A.J. and J.S. Craigie, 1973. Handbook of Phycological Methods: Physiological and Biochemical Methods. Cambriage University Press, pp: 506-512.

Hoek, C., D.G. Mann and H.M. Johns, 1995. Algae-An Introduction to Phycology. Cambridge University Press.

Kessler, E., 1982. Chemotaxonomy in the Chlorococcales. In: Round, F.E. and D.J. Chapman (Eds.). Prograss in Phycological Research. Elsevier Amesterdam, 1: 111-135.

Kessler, E. and V.A.R. Huss, 1992. Comparative physiology and biochemistry and taxonomic assignment of the *Chlorella* (Chlorophyceae) strains of the culture collection of the university of texas at austin. J. Phycol., 28: 550-553.

Kim, J.K., 1998. Continuous fermentation of *Kluyeeromyces fragilis* for the production of a highly nutritious protein diet. Aquacul. Eng., 18: 41-49.

Kobayashi, M. and S.I. Kurata, 1978. The mass culture and cell utilization of photosynthetic bacteria. Process Biochem., 13: 27-30.

Li, H.B., F. Chen, T.Y. Zhang, F.Q. Yang and G.Q. XU, 2001. Preparative isolation and purification of lutein from the micro algae *Chlorella vulgaris* by high speed countercurrent chromatography. J.A Chromatogr., 905: 151-155.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.L. Randall, 1951. Protein measurement with the follin phenol reagent. J. Biol. Chem., 193: 265-273.

Richmond, A., 1983. Handbook of Microalgal Mass Culture. CRC Press, Inc. Boca Raton, Florida.

Rumsey, G.L., 1981. Dairy processing wastes as a replacement protein source in diet of rainbow trout. The Progressive Fish-Culturist, 43: 86-88.

Shihira, I. and R.W. Krauss, 1965. *Chlorella* Physiology and Taxonomy of Forty-one Isolates. University of Maryland, College Park, Maryland, pp: 1-92.

Thanikachalam, A. and M. Rangarajan, 1986. Protein enrichment of rice straw by fermentation with *Cellulomonas* sp., Agric. Wastes, 15: 215-224.

Watanabe, Y., N. Ohmura and H. Saiki, 1992. Isolation and determination of cultural characteristics of micro algae which functions under CO_2 enriched atmosphere. Energy Conversation Manage., 33: 545-552.