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A NEW AND SIMPLE MEDIUM FOR PHYTOPLANKTON CULTURE

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Abstract

Results of the study using a simple medium with a higher concentration of phosphate, developed for isolating unicellular algae from Calicut region are presented in this paper. The new medium is found to be very simple for large scale culture of phytoplankton to develop larval feed in hatcheries. The medium is prepared by dissolving 350 mg of potassium di hydrogen phosphate and 900 mg of potassium nitrate in 125 ml distilled water. One ml of this solution is added to a litre of boiled and filtered sea water. A stock solution of vitamin is made by dissolving 5 mg of B_{12} and 95 mg of B_1 in 100 ml of distilled water and one ml of this solution is added to each litre of the nutrient mixed sea water. *Chromulina* sp. *Pavlova* sp. and *Chlorella* sp. are inoculated in 250 ml flasks containing the new medium and is simultaneously compared with another set of flasks containing Walne's medium under controlled conditions of light and at a temperature ranging from 28 to 30°C. After every twenty four hours, cell counts are made and it is observed that rapid multiplication of cells occurs during the initial period itself and the exponential phase is continued from the sixth day upto 12th day with similar results in both the media. The results with different concentrations of the nutrients in the new medium are also presented in the paper.

Introduction

Aquaculture in Marine, Brackishwater and in Fresh water has made paid progress in India during the past decade (MBAI, 1980 CMFRI, 1988) and Sinha and Srivatsava, 1991) and the efforts for seed production of culturable species through hatcheries also have increased

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subsequently to meet the requirements of farmers. It is needless to state that to economise the hatchery production, the essential operational inputs are to be minimised for all stages of hatchery programmes including feed development by adopting low-cost productions.

It is well known that schreiber's solution, Miquel's solution Convey or Walne's medium, TMRL medium, PM solution etc. are the culture media conventionally used by various laboratories for culture of phytoflagellates, diatoms etc. and their composition are given by Gopinathan (1982).

The Central Marine Fisheries Research Institute has initiated a project to establish a hatchery for mussel seed production in 1989 at Calicut and the initial investigations were carried out to develop hatchery related technology to culture unicellular phytoflagellates on a large scale for feeding the larvae of mussels, brood-stock etc. Instead of using conventional medium, it was necessary to make new combinations of chemicals, minimising the number of ingredients, which is found to be highly useful for large scale culture of unicellular algae and the results are presented in this paper.

Materials and Methods

Three experiments are conducted by preparing the new nutrient media using potassium nitrate and potassium di hydrogen phosphate in different proportions along with vitamin B. The third experiment was conducted without adding Vitamin B.

In the first experiment 900 mg of potassium nitrate and 350 mg of potassium di hydrogen phosphate are dissolved in 125 ml distilled water. One ml of this solution is added to a litre of boiled and filtered sea water. A stock solution of vitamin is made by dissolving 5 mg of B_{12} and 95 mg of B_1 in 100 ml of distilled water and one ml of this solution is added to each litre of the nutrient mixed sea water. *Chromulina* sp. *Pavlova* sp. and *Chlorella* sp. are inoculated in 250 ml flasks containing the new medium and is simultaneously compared with the results of another set of flasks containing Walne's medium and the above algae respectively under controlled conditions of light and at a range of water temperature 28 to 30°C. Cell counts are made after every 24 hours.

The second experiment was conducted using the nutrient medium prepared by dissolving 900 mg of potassium nitrate and 180 mg of potassium di hydrogen phosphate in 125 ml distilled

water. Vitamin B stock solution is also prepared separately as described above. One ml of the nutrient medium and one ml of Vitamin B are added in every litre of boiled and filtered sea water and the experiment is repeated as in the first experiment using the algae and the Walne's medium.

The third experiment is carried out using the second medium and without Vitamin B. For all the experiments the flasks are plugged with cotton for proper aeration.

As the new medium is found to be suitable with the proportion of chemicals in the first experiment as well as in the third experiment without vitamin B, the above media are used for developing the mixed culture of algae (Chromulina sp., Pavlova sp. and chlorella sp.) in large scale for feeding larvae and for developing brood stock for hatchery purpose.

Results

The results of the first experiment by using Walne's medium and the new medium are presented in Table I. Even though cell multiplication started from the first day onwards after a slow multiplication process upto the 5th day, rapid growth was observed from the 6th day to 12th day and the rate decreased from the 13th day onwards in the case of *Chromulina* sp. and *Pavlova* sp. But in the case of *Chlorella* sp. after the 14th day only the decrease in cell division was observed. However, the results were found to be comparatively similar in both the media.

In the second experiment rapid multiplication was observed from the fifth day onwards upto the eighth day and it continued to slow down for all the species of algae and the results are presented in Table II. However the experiment was discontinued after the 12th day because of the downward trend of cell multiplication in case of all species of algae. The results of the third experiment using the new medium with low concentration of phosphate and without Vitamin B are given in Table III. The progress of cell multiplication is found to be very uniform from the beginning with a very fast rate of division from the sixth day with similar results in both the media. From the 12th day onwards a decreasing trend is observed and the experiment is continued upto the 16th day. The results of the three experiments show that the new medium with less number of chemicals can be successfully used for culturing phytoflagellates and the results are comparably similar to Walne's medium having many chemicals. The medium is used for subsequently raising of the mixed culture with encouraging results. By periodically changing the medium, the stocks of monoculture of mixed culture can be maintained continuously.

Table - I

GROWTH OF ALGAE IN WALNE'S MEDIUM AND NEW MEDIUM EXPT.I

(Number of cells/ml in lakhs)

No. of days after inoculation	Chromulina sp.		Pavlova sp.		Chlorella sp.	
	Walne's medium	New medium	Walne's medium	New medium	Walne's medium	New medium
1	1.4	0.8	2.2	2.0	1.1	0.98
2	12.7	12.9	5.5	5.1	20.2	18.9
3+	-	-	-	-	-	-
4+	-	-	-	-	-	-
5	86.0	88.8	70.7	81.5	136.5	143.0
6	186.3	184.8	166.8	162.8	441.3	460.4
7	519.5	529.5	284.2	292.2	852.8	857.8
8	992.4	1000.5	558.2	592.3	1704.9	1688.8
9	916.4	1026.5	700.3	714.3	1720.8	1708.8
10	994.9	1008.5	762.3	612.3	1740.8	1740.8
11+	-	-	-	-	-	-
12	988.4	988.9	770.3	754.3	1921.5	1978.9
13	434.3	537.3	400.4	346.3	2195.0	2157.0
14	704.3	692.3	696.3	652.3	1436.7	1562.7
15	470.2	530.2	460.2	528.2	972.4	856.4
16	83.1	125.2	170.8	165.3	514.5	436.4

+ Not observed due to holiday.

Table - II

GROWTH OF ALGAE IN WALNE'S MEDIUM AND NEW MEDIUM. EXPT.
(Number of cells/ml in lakhs)

No. of days after inoculation	Chromulina sp.		Pavlova sp.		Chlorella sp.	
	Walne's medium	New medium	Walne's medium	New medium	Walne's medium	New medium
1	0.44	0.39	0.45	0.47	0.66	0.64
2	1.0	1.5	1.0	0.98	1.1	1.3
3	1.5	1.1	2.7	2.8	28.3	29.2
4+	-	-	-	-	-	-
5	963.9	1040.0	268.8	223.1	181.2	228.9
6	1789.8	1859.9	460.4	457.9	427.9	471.4
7	1755.8	1999.9	472.4	521.8	648.8	871.7
8	1625.6	2052.0	1401.4	1818.8	1998.9	2206.2
9+	-	-	-	-	-	-
10	391.7	538.6	312.6	372.2	1264.3	1651.7
11	134.5	215.6	0.7	0.8	2965.9	1438.4
12	69.3	93.4	1.4	2.2	1292.3	1031.0

+ Not observed due to holiday.

Table - III

GROWTH OF ALGAE IN WALNE'S MEDIUM AND NEW MEDIUM EXPT. 3
(Number of cells/ml in lakhs)

No of days after inoculation	Chromulina sp.		Pavlova sp.		Chlorella sp.	
	Walne's medium	New medium	Walne's medium	New medium	Walne's medium	New medium
1	0.6	0.4	1.4	1.3	1.3	1.4
2	1.2	1.2	2.8	2.7	3.1	3.1
3	293.4	294.2	278.6	266.1	371.5	327.1
4	294.6	273.1	415.3	367.2	425.9	400.8
5	657.7	582.5	682.7	616.6	1040.0	1199.2
6	1431.4	1134.1	1082.1	963.9	1497.5	1575.6
7	918.9	1035.4	1839.8	1733.7	3407.4	3331.3
8	3755.7	3555.6	3739.7	3763.8	6106.1	6382.4
9	1718.2	1688.2	1990.8	1738.2	2495.9	2448.4
10	2216.4	2188.4	2608.5	2591.0	3291.3	2892.2
11	1260.6	1298.6	1472.7	1164.3	1782.9	1802.9
12	587.6	603.6	666.7	594.6	1027.0	882.9
13	215.4	229.9	295.1	273.5	562.6	385.2
14	87.1	115.7	117.9	114.9	230.4	156.1
15	35.4	51.6	52.0	33.4	62.2	29.7
16	2.8	3.2	4.4	3.2	55.8	33.2

It is observed that for all the conventional media given elsewhere in this paper either potassium nitrate and sodium phosphate or sodium nitrate and potassium phosphate are used along with growth promoting chemicals and vitamin without using potassium salt alone for both nitrates and phosphates. In the new medium potassium salts are used for both nitrate and phosphate and hence the concentration of potassium in the medium will be normally high. As the medium contains only two chemicals it is easy for any laboratory to prepare the same in bulk quantity and the cost of production also is very low. The suitability of this medium of large scale culture of mixed phytoflagellates is also found to be highly encouraging and this could be used in any hatchery to culture larval feed.

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