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RESEARCH ARTICLE

ALGAE BIOTECHNOLOGY

ROLE OF THIAMINE AND ITS MOIETIES IN GROWTH RATE OF DIATOM SP.

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ABSTRACT

Diatom sp isolated from the coastal water around Chennai, and Tamilnadu was made as unialgal and investigated for growth. The culture is grown aseptically in F/2 medium under constant temperature and light. This study focus on the influence of thiamine and its moieties in Diatom sp. Thiazole and pyrimidine are two thiamine moieties were used as vitamin source in diatom culture medium separately. Three diatom cultures were used for this study. All diatom cultures were grown separately in (A) Medium.1 -F/2 medium with thiamine. (B)Medium.2 -F/2 medium with thiazole. (C)Medium.3 -F/2 medium with pyrimidine. The result shows that Diatom *Navicula* sp reaches maximum growth rate thiazole medium and Diatom *Cymbella* sp reaches maximum growth rate pyrimidine medium and Diatom *Cyclotella* sp. reaches maximum growth rate of both moiety of thiamine control medium. From this research we conclude that *Navicula* sp need only thiazole and *Cymbella* sp need only pyrimidine and *Cyclotella* sp need both thiamine moieties for their normal growth.

KEYWORDS

Algae, Thiamine, F/2 medium, Diatom, Thiazole and Pyrimidine.

INTRODUCTION

Diatoms are a major group of algae, and are one of the most common types of phytoplankton. Most diatoms are unicellular. A unique characteristics feature of Diatom cells is that they are encased within unique cell wall made of silica. Most Diatoms are unicellular, although they can exist as colonies. Diatoms are a widespread group and can be found in oceans, fresh water and in soils. Diatoms belong to a large group of algae called the heterokonts that includes both autotrophs and heterotrophs.Diatoms yellowish brown chloroplasts are typical of heterokonts, with four membranes and containing pigments such as the carotenoids and fucoxanthine. Diatoms can be grown both on agar gels and in liquid. Many species are easy to grow and that the same time it easily contaminated with other algae. Before isolation, it may be necessary to grow a mixed culture from materials collected from nature in order to obtain a large number of cells or it may be possible to pick out a small number of cells from natural population and grow them in a defined culture medium. The initial inoculums can be streaked on agar plates [1-2% made up in culture medium]. The individual colonies can then be removed with a wire loop. The cultures can be maintained unialgal in various light/dark cycles and at various temperatures. Aeration will be required, if large volume of cultures is maintained. Bacterial contaminants can be eliminated by the addition of appropriate antibiotics of the media.

Thiamine is a colourless compound .Its structure contains a pyrimidine ring and a thiazole ring linked by a methylene bridge. Thiamine is soluble in water, methanol, and glycerol and practically insoluble in acetone, ether, chloroform, and benzene. It is stable at acidic pH, but is unstable in alkaline solutions. Thiamine is unstable to heat, but stable during frozen storage. It is unstable when exposed to ultraviolet light and gamma irradiation. Thiamine also plays a pivotal role in intermediary carbon metabolism. The active form of the vitamin is thiamine pyrophosphate (TPP), which is essential for all organisms. The cofactor associates with a number of enzymes involved in primary carbohydrate and branched-chain amino acid metabolism. Thiamine consists of a thiazole and a pyrimidine moiety, which are produced in separate branches of the biosynthetic pathway before being coupled together to produce thiamine phosphate. This is then further phosphorylated to produce the active cofactor TPP. Thiamine was the first vitamin found to be an algal growth factor. Early studies on the specificity of this requirement showed that in some cases thiamine auxotrophy could be relieved by addition of the thiazole moiety to the growth medium, in others cases the pyrimidine moiety was sufficient, while in the final group of auxotrophs the full thiamine molecule was essential for growth. These studies shows that in algae the thiamine biosynthetic pathway follows the same general pattern as in other organisms, with two separate branches to make each of the moieties, which are then combined together to make thiamine. Furthermore, the presence of some parts of the pathway in thiamine auxotrophs suggests that they require the vitamin because they have lost one or more of the essential genes involved in its biosynthesis [1].

The requirement for thiamine by so many disparate algae indicates that the vitamins are available in the environment and that mechanisms exist for their uptake into algal cells. This vitamin is water soluble and comparatively stable, suggesting that they

can be rescued by salvage. Indeed, thiamine-scavenging pathways are known in animals, fungi, and eubacteria. These vitamins are cofactors for a limited number of enzymes and are thus required in small quantities, reducing the pressure on biosynthetic flux and making salvage a viable option. However, the uptake of these compounds is not as simple as it may at first seem because their concentration in the natural environment is extremely low. Indeed, the minute amount of these organic micronutrients has made them difficult to measure the concentrations of thiamine in the natural environment are below that normally required in culture, with thiamine levels typically varying between 8 and 15 ng/liter[4] .

MATERIALS AND METHODS

The algae Diatom sp. upon which work has been carried out was isolated in Department of Biotechnology, School of life sciences, Vels University, Chennai, from the coastal water around Chennai, and Tamilnadu. Three algae was identified and isolated from sample and grown in F/2 culture medium. Isolation of pure culture is an important preliminary to the study. Since it is possible that different physiological races of a species of algae may exist, it is desirable that the cultures used should have originated from a single individual. Such unialgal cultures of *Cymbella* sp., *Navicula* sp., *Cyclotella* sp., were obtained by isolating an individual with a sterile serial dilution followed by agar streaking method and pure colonies were isolated. The culture was examine under the low power microscope and inoculated into sterile f/2 culture medium. Cultures obtained by above method were still contaminated with bacteria. To obtain pure culture free from bacteria has been difficult and this was achieved by sub culturing on agar plates. A small portion of algae culture was immersed in chlorine water of a concentration of 25mg per 100 ml for 5 minutes and centrifuges the sample at 10,000 rpm at 30°C for 5 minutes in cooling centrifuge. After centrifuge the supernatant was discarded and the pellet washed in sterile water and the pellet was transfer in freshly prepared culture medium. All the anexic cultures used in the subsequent work were grown from this culture.

The chemicals used were of analytical quality. The medium was sterilized at 120°C for 15 minutes in the autoclave and allow standing at least 3 hours before inoculation and its pH was checked for 7.8. Conical flask of 1000ml capacity, plugged with cotton wool, was used throughout this work. The flasks were cleaned with chromic acid and rinsed with distilled water before use. Culture chamber was maintaining a constant temperature below 30°C. The temperature remained at 21°C for most of the time. Illumination was for a period of about 12 hours per day. Stock cultures were maintained on agar slants in test tubes. Before using a culture for inoculums, it was thoroughly examined for contaminants. A portion of material from young stock culture was shaken with sterile medium in a sterile flask closed with tightly fitting cotton plug for 15 minutes. The heavier materials were allowed to settle and after decantation portions of 50ml of the suspension were used as inoculums. The suspension prepared in this manner was found to be sufficiently uniform for all ordinary purpose. For the contents of a culture flask were to analyzed, the alga was first detached from the sides of the flask by means of vigorous shaking then the algae and medium were separated by centrifugation at 10,000 rpm. The algae were washed with distilled water and the pellet was added to the medium. The bio mass was dried for analysis by evaporation in hot plate at 75°C.

Inoculum preparation

The microalga, *Cymbella* sp., *Navicula* sp., *Cyclotella* sp., was inoculated in three F/2 medium. Medium.1 -F/2 medium with thiamine [200mg/L]. Medium.2 -F/2 medium with thiazole [200mg/L]. Medium.3 -F/2 medium with pyrimidine [200mg/L] and the culture was incubated for 21days at 21ºC in a thermo-statically controlled room and illuminated with cool inflorescence lamps at an intensity of 2000 lux in a 12: 12 h light dark regime.

Growth measurement

Growth was measured by counting cells using a haemocytometer (Neubauer, improved) throughout the study period. The culture was sampled once in every 5 days and the cell numbers were measured using a haemocytometer (Neubauer, improved).

Analytical procedure

Chlorophyll was estimated using the extinction coefficients given by Jeffery and Humphrey (1975)[10]. Total carbohydrates by anthrone method and total lipid content according to Bligh and Dyer (1959)[2]. pH were measured using digital pH meters, throughout the study period. The bacterial cell numbers were measured by standard plate count method (pour plate technique).

RESULTS

Three Diatom sp. were cultivated in three different F/2 medium. Medium.1 -F/2 medium with thiamine [200mg/L]. Medium.2 -F/2 medium with thiazole [200mg/L]. Medium.3 -F/2 medium with pyrimidine [200mg/L] was prepared and *Cymbella* sp., *Navicula* sp., *Cyclotella* sp., was inoculated and it was cultured for 21 days at 21°C with 2000k lux with frequent sampling. Effect of thiamine and its moieties in *Cyclotella* sp are represented in Table: 1. Effect of thiamine and its moieties in *Navicula* sp are represented in Table: 2. Effect of thiamine and its moieties in *Cymbella* sp are represented in Table: 3.

Growth measurement

The cultures were sampled once in every 5 days and the cell numbers were measured using haemocytometer. *Cyclotella* sp: on the final day there was 1300 $\times 10^{-4}$ cells/mL for the medium-1 and 1000 $\times 10^{-4}$ cells/mL, 900 $\times 10^{-4}$ cells/mL for medium-2 and medium-3 respectively. *Navicula* sp: on the harvesting day there was 1100 $\times 10^{-4}$ cells/mL for the medium-2 and 700 $\times 10^{-4}$ cells/mL, 800 $\times 10^{-4}$ cells/mL for medium-3 and medium-1 respectively. *Cymbella* sp: on the last day there was 900 $\times 10^{-4}$ cells/mL for the medium-3 and 500 $\times 10^{-4}$ cells/mL, 400 $\times 10^{-4}$ cells/mL for medium-1 and medium-2 respectively. Graph :1 represented the effect of thiamine and its moieties in growth rate of Diatom sp.

Measurement OF pH

Samples were drawn from all nine cultures on a routine basis. pH were measured daily and the results indicate that the pH level raise from 7.5 on day 1 to 9.5 on day 21. For all the 21 days, the increase in pH was gradually.

Estimation of chlorophyll

All culture was sampled on 21st day and harvested by centrifugation. Chlorophyll were analysed by Spectrophotometric method. Chlorophyll level was in maximum on the 19th day and decreased gradually on subsequent days.

Cyclotella sp: on the 19th day there was 5.9 pg/ cell-1 for the medium-1 and 3.0 pg/ cell-1, 2.5 pg /cell-1 for medium-2 and medium-3 respectively. *Navicula* sp: on the 19th day there was 7.3 pg/ cell-1 for the medium-2 and 6.0 pg/ cell-1, 4.0 pg/ cell-1 for medium-1 and medium-3 respectively. *Cymbella* sp: on the 19th day there was 6.3 pg/ cell-1 for the medium-3 and 4.0 pg/ cell-1, 2.3 pg/ cell-1 for medium-1 and medium-2 respectively. Effect of thiamine and its moieties in chlorophyll of Diatom sp are represented in Graph :2.

Estimation of carbohydrates

Culture samples were collected on 21st for the analysis of carbohydrate. The results show that the *Cyclotella* sp: on the 19th day there was 0.0120g/ L for the medium-1 and 0.0090g/ L, 0.0070g/ L for medium-2 and medium-3 respectively. *Navicula* sp: on the 19th day there was 0.0160g/ L for the medium-2 and 0.0090g/ L, 0.0080g/ L for medium-1 and medium-3 respectively. *Cymbella* sp: on the 19th day there was 0.0150g/ L for the medium-3 and 0.0100g/ L, 0.0087g/ L for medium-1 and medium-2 respectively. Effect of thiamine and its moieties in carbohydrate of Diatom sp are represented in Graph :3.

Estimation of lipids

All nine Culture samples were collected on 21st for lipid analysis. The results show that the *Cyclotella* sp: on the 19th day there was 0.0300g/ L for the medium-1 and 0.0200g/ L, 0.0200g/ L for medium-2 and medium-3 respectively. *Navicula* sp: on the 19th day there was 0.0219g/ L for the medium-2 and 0.0120g/ L, 0.0110g/ L for medium-1 and medium-3 respectively. *Cymbella* sp: on the 19th day there was 0.0360g/ L for the medium-3 and 0.0250g/ L, 0.0200g/ L for medium-1 and medium-2 respectively. Effect of thiamine and its moieties in lipids of Diatom sp are represented in Graph :4.

DISCUSSION

The effects of thiamine and its moieties in F/2 culture medium on growth kinetics and fatty acid production of three Diatom sp. were investigated. The requirement of thiamine and its moieties was examined on C*ymbella* sp., *Navicula* sp., *Cyclotella* sp., the thiamine molecule is composed of a thiazole and pyrimidine moiety. These moieties can often replace intact thiamine. The thiamine requirement is satisfied by the thiazole moiety alone in few species, by the pyrimidine moiety in few species, some species require both moieties. In our study *Cyclotella* sp., is well active in growth in F/2 medium containing thiamine, *Navicula* sp., is shows maximum growth in F/2 medium containing only thiazole moiety and *Cyclotella* sp., in F/2 medium containing only pyrimidine moiety shows maximum growth rate. pH study, showed as steady increase in pH reaching to around 9 .5 at the end of the study in all nine cultures. Among the chlorophyll pigment, Carbohydrate and lipids in *Cyclotella* sp in medium-1, *Navicula* sp in medium-2 and *Cymbella* sp in medium-3 was the highest. The *Cyclotella* sp are quite satisfied in thiamine F/2 medium. So their response to thiazole and pyrimidine medium can be regarded as simple. Whereas in *Navicula* sp shows maximum responses towards thiazole moiety when compare to pyrimidine and thiamine F/2 medium. On the other hand the *Cymbella* sp shows maximum benefits towards pyrimidine F/2 medium. In *Cyclotella* sp the both thiamine moiety are utilized equally and both moieties are required by the organism for their normal growth. In *Navicula* sp the thiazole moiety only require for the normal growth when compare to pyrimidine and in Cymbella sp the pyrimidine moiety used frequently by the organism when compared to thiazole. It suggests either that thiamine has a different function according to whether the requirements are met by one half of the vitamin or by the other or, alternatively, that the function of pyrimidine and thiazole are divorced from each other and that the function of thiamine here is merely to supply either thiazole or pyrimidine as the case may be. Which alternative is biochemically less improbable it is difficult to say. On the other hand, it is possible that thiazole is merely more liable than pyrimidine.

Table 1
Effect of Thiamine and its moieties in Cyclotella sp.

	Cyclotella sp		
Parameters	Medium -1 [Thiamine]	Medium-2 [Thiazole]	Medium-3 [Pyrimidine]
Cell volume [10^{-4} cells/mL]	1300	1000	900
Chlorophyll [pg/ cell-1]	5.9	3.0	2.5

Carbohydrate[g/L]	0.0120	0.0090	0.0070
Lipids[g/L]	0.0300	0.0200	0.0200

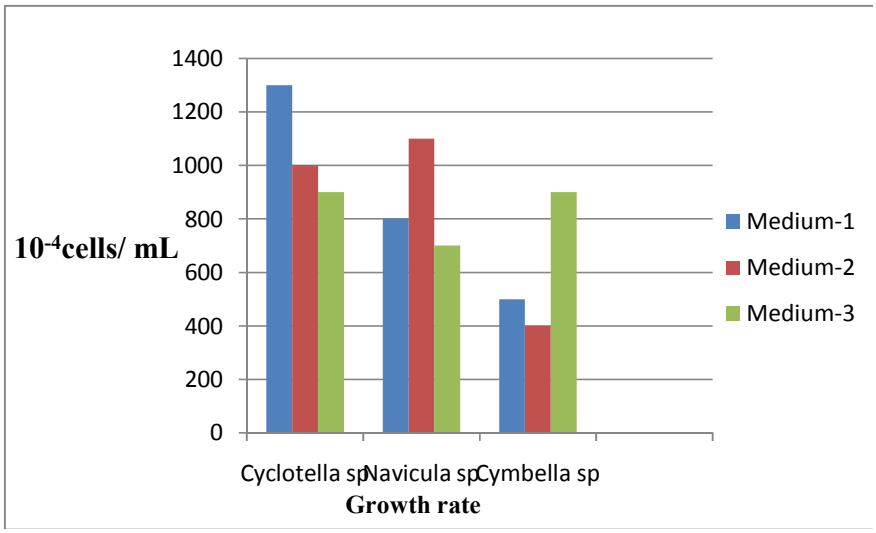
Table 2
Effect of Thiamine and its moieties in Navicula sp.

	Navicula sp		
Parameters	Medium -1 [Thiamine]	Medium-2 [Thiazole]	Medium-3 [Pyrimidine]
Cell volume [10^{-4} cells/mL]	800	1100	700
Chlorophyll [pg/ cell-1]	6.0	7.3	4.0
Carbohydrate[g/L]	0.0090	0.0160	0.0080
Lipids[g/L]	0.0120	0.0219	0.0110

Table 3
Effect of Thiamine and its moieties in Cymbella sp.

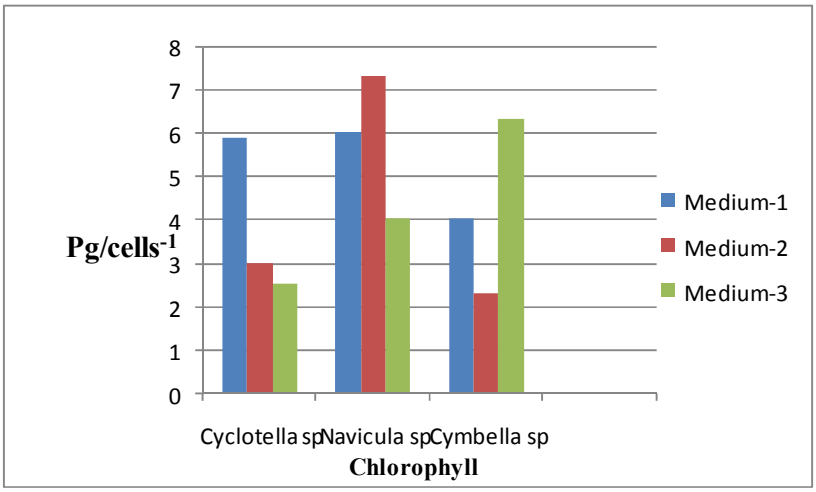
	Cymbella sp		
Parameters	Medium -1 [Thiamine]	Medium-2 [Thiazole]	Medium-3 [Pyrimidine]
Cell volume [10^{-4} cells/mL]	500	400	900
Chlorophyll [pg/ cell-1]	4.0	2.3	6.3
Carbohydrate[g/L]	0.0100	0.0087	0.0150
Lipids[g/L]	0.0250	0.0200	0.0360

Graph 1
Effect of Thiamine and its moieties in Growth rate of Diatom sp



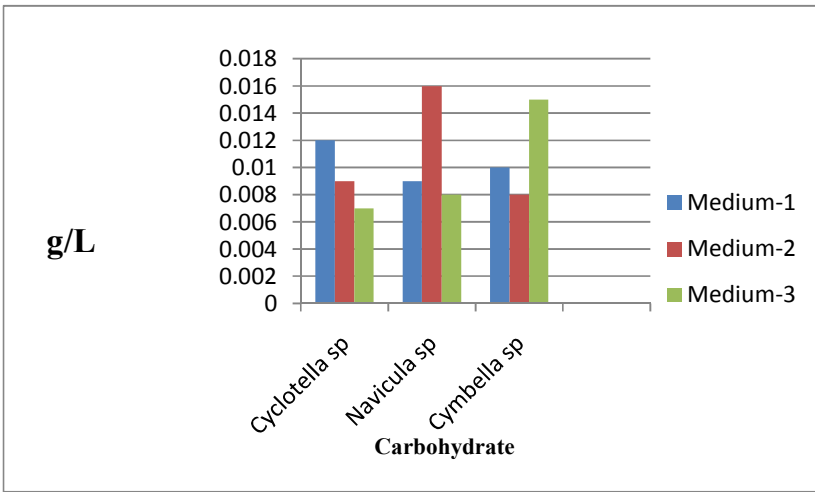
Graph 2

Effect of Thiamine and its moieties in Chlorophyll of Diatom sp

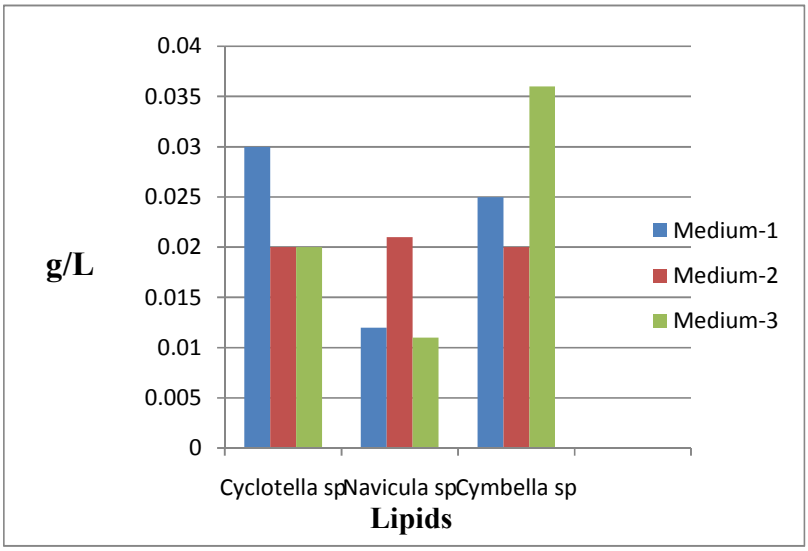


Graph 3

Effect of Thiamine and its moieties in Carbohydrates of Diatom sp



Graph 4
Effect of Thiamine and its moieties in Lipids of Diatom sp



CONCLUSION

From this study we conclude that the result shows Diatom *Navicula* sp reaches maximum growth rate in thiazole medium and Diatom *Cymbella* sp reaches maximum growth rate in pyrimidine medium and Diatom *Cyclotella* sp. reaches maximum growth rate of both moiety of thiamine control medium. From this research we conclude that *Navicula* sp need only thiazole and *Cymbella* sp need only pyrimidine and *Cyclotella* sp need both thiamine moieties for their normal growth.

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