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Research Article

A study on *Ochromonas* sp. as an alternate microalgal biomass for the synthesis of proteins and carotenoids

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Abstract
Microalgae are a good source of antioxidants and natural bioactive compounds utilized in the pharmaceutical and food industries.The present study aimed to explore *Ochromonas gloeopara* as an alternate microalgal biomass for synthesising proteins and carotenoids.The freshwater samples of microalgae collected from a lake had microalgae and macroalgae that feed on microalgae. This sample was further subcultured in BG-11 media with 20nM phosphate to inhibit the growth of predator species. The isolated microalgae were identified as *Ochromonas cf. gloeopara* by 18S rRNA sequencing. A comparison of growth characteristics and protein production by the isolate with *Chlorella vulgaris* indicated that *O. gloeopara* had a uniform growth rate and a better protein production of 33.83mg/g. Carotenoid production was found to be 424.64 µg/g and 263.87 µg/g dry biomass *by O. gloeopara* and *C. vulgaris,* respectively. Thin Layer Chromatography analysis revealed three types of carotenoids: β-carotene, astaxanthin mono and diesters produced by *C. vulgaris*, whereas *Ochromonas* produced only β-carotene. Physical parameter studies revealed that the optimum growth condition for *C. vulgaris* was at 1% salinity and pH 7, but it had a better carotenoid production at 0.5% salinity. *O. gloeopara* had better growth and production of carotenoids at 0% salinity and pH 7. These carotenoids and proteins can be used in various food and pharma industries.

Keywords: β-carotene, Carotenoid, *Chlorella vulgaris, Ochromonas cf. gloeopara,* Proteins

INTRODUCTION

Microalgae are a broad class of organisms classified as prokaryotes or eukaryotes and unicellular, siphonaceous, filamentous, or multi-cellular. However, they are all photosynthetic microorganisms. They are considered a rich source of bioactive and health-promoting substances such as vitamins, proteins, and carotenoids (Scoglio *et al*., 2024; Y. Wang *et al*., 2021). Due to their great metabolic flexibility, adaptability to different culture conditions, and potential for fast growth, studies on their use as a source of biologically valuable compounds are rapidly increasing. There are approximately 200,000 species of microalgae and they are the largest primary producers (Hamid *et al*., 2025). They are exploited for commercial purposes to produce various bioactive compounds that find applications in the pharmaceutical, cosmeceutical, nutraceutical, biofertilizers, feed, and biofuel industries (Asadi *et al*., 2020; Dolganyuk *et al*., 2020). In Europe, microalgae and seaweed are currently produced by about 420 companies across 23 countries; 46% of these companies produce Spirulina 36% produce seaweed, and 10% produce microalgae. The remaining 8% produces Spirulina and several other microalgae. The market for algae is expected to reach EUR 1131 million by 2027, from EUR 594 million in 2018 (Mendes *et al*., 2022).

Chlorella is commonly found in soil, marine, and freshwater. The genus of *Chlorella* comprises 37 taxonomically acknowledged species. Due to their rapid growth

and high photosynthetic ability, they are considered a promising biological resource(Schüler *et al*., 2020). The industrial applications of *Chlorella vulgaris* are very broad because of its ability to produce carotenoids, which are used to produce nutraceuticals and feed (Chia *et al*., 2019; Jo *et al*., 2020). There are several dietary sources of carotenoids, predominantly plants and animals (Mapelli-Brahm *et al*., 2020). There is growing attention to other dietary sources of carotenoids, Including macroalgae and microalgae (Deamici *et al*., 2025; Meléndez-Martínez *et al*., 2022).

The microalgae *Ochromonas*, the ubiquity, ecological significance, and nutritional versatility of *Ochromonas* species make them ideal model candidates for research on nutrient production. The mixotrophic chrysophytes of the genus *Ochromonas* are found mostly in freshwater, brackish, marine, and even extreme environments like hypersaline ponds (Lie *et al*., 2017). They use a wide variety of feeding methods. It has been found that many *Ochromona*s species, such as *Ochromonas danica*, are phagotrophic. They can grow in total darkness, provided there is bacterial prey present. Some species of *Ochromonas* are exclusively dependent on light, and others rely largely on phototrophy(Lie *et al*., 2018). In addition, *Ochromona*s species have been shown to grow exclusively on phenol as a carbon source and to be osmotropic, albeit only at elevated levels of labile organic compounds(Lie *et al*., 2017).

Carotenoids are lipophilic pigments that appear in different colors, such as red, orange, and yellow. These pigments are abundantly found in nature and over 700 different compounds have been identified. Non-chlorophyll pigments 4are crucial in light harvesting and photoprotection in all photosynthetic organisms. Primary and secondary carotenoids are the two categories of carotenoids. Primary carotenoids are essential to the organism's survival because they directly contribute to photosynthesis. Lutein, α-carotene, and β-carotene are the most common primary carotenoids(Dalal and Siddiqui, 2025). Secondary carotenoids are compounds produced by carotenogenesis when the organism is subjected to a particular stimulus. The most widely used carotenoids in the global market are lutein, canthaxanthin, lycopene, zeaxanthin, β-carotene, and astaxanthin (Hamidi *et al*., 2019). Carotenoids have numerous applications across the pharmaceutical and nutraceutical sectors. The demand for carotenoids has surged worldwide due to their increasing significance in enhancing food quality. They are also used in poultry and fish feed. They are used as supplements in functional foods as they are a good source of nutrients, antioxidants, and vitamins (Pashkow *et al*., 2008; Wang *et al*., 2022). They can be used as an alternative to fish oils (Novoveská *et al*., 2019).Most of the research on carotenoid compounds has focused on how they can protect against and prevent various chronic illnesses, including cancer, diabetes, metabolic syndrome, and cardiovascular disease. However, recent studies indicate that carotenoids might also be crucial in the management of several different illnesses (Sathasivam and Ki, 2018).

Red algae contain a pigment called phytocyanin, which has anti-inflammatory and antioxidant properties and is used in food and cosmetic products. Astaxanthin has significant antioxidant activity, which benefits human health by providing the carotenoid with anti-inflammatory and UV light-protective qualities(Aslam *et al*., 2019; Gokbulut, 2024). These new findings make beta-carotene much more valuable and may lead to an increase in demand for it. Few food coloring products are made from microalgae. The present study aimed to determine the carotenoid and protein production by *C. vulgaris* and microalgae isolated from lake water samples.

MATERIALS AND METHODS

Isolation and screening of microalgae

The water sample collected at 13.0360° N, 77.6586° E was serially diluted and used as inoculum for BG-11 media. The dilution with ample growth after 5 days was used for spread plating on 1% BG-11 agar. When colonies grew on the plate, one colony was selected and suspended in BG-11 media and allowed to grow for 5 days. This was inoculated in fresh BG-11 media with 20 mM phosphate to inhibit the growth of predator species. (Toda *et al*., 2021). After 7 days, the microscopic examination of broth was done at 100x to determine the presence of microalgae. After centrifuging the broth for ten minutes at 8000 rpm, the pellet was used to extract DNA followed by 18S rRNA sequencing, where the sample was processed and subjected to the “PCR Sanger Sequencing” method using the following primers

Forward primer: 5’-CGGACGGGTGAGTAACGCGTGA-3’
Reverse primer: 5’-GACTACAGGGGTATCTAAT CCC TTT-3’

Using Nucleotide BLAST, the obtained data sequence was analyzed, and the phylogenetic tree was generated. A standard culture of *Chlorella vulgaris* obtained from Biopol Bioscience Pvt.Ltd., Bangalore was used as a reference for further studies.

Growth Kinetic Studies

5% sample was inoculated in BG11 media, this culture was studied daily, where 3ml of the cultured sample was drawn out periodically at an interval of 24 hours and subsequently, The OD of the sample was taken at 680 nm, where OD of one corresponds to $4.858x10^4$ cells/ml (Christwardana and Hadiyanto, 2017).

The growth rate of the sample was determined using the formula:1

Fig. 1. *Ochromonas cf. gloeopara* with *Poterioochromonas malhamensis* at 100x

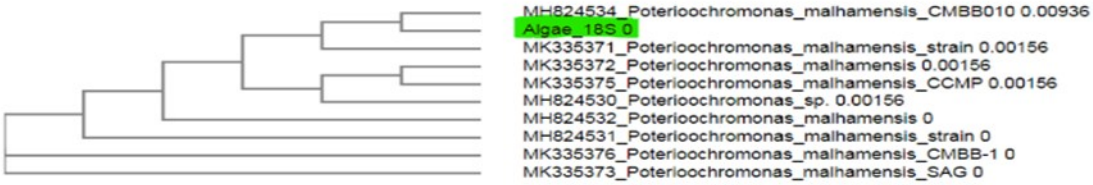

Fig. 2. *Phylogenetic tree showing the relationship of Poterioochromonas malhamensis with closely related taxa based on partial 18S rRNA gene sequence*

$\mu = 3.3 (\log_{10}N - \log_{10}N_0)/t$ (1)

Where μ: growth rate N and N_0: final and initial growth, respectively, t: time (Singh *et al*., 2019a).

Estimation of proteins and carotenoids

Lowry's method was used to estimate the amount of protein present in the samples, and the optical density was measured at 660 nm (Novoveská *et al*., 2019). To estimate the carotenoid content, 2 ml of the sample was taken and centrifuged for 10 minutes at 8000 rpm. The pellets were closely resuspended in 2 ml acetone and kept in the water bath for 2 hours with agitation every 10 minutes. The optical density of the solution was taken at different wavelengths 660, 645, and 470 nm. The carotenoid content was determined using the following formulas:

Chlorophyll *a* (μg/mL) = (11.24*A660) - (2.04*A645) (2)

Chlorophyll *b* (μg/mL) = (20.13*A645) - (4.19*A660) (3)

Carotenoids (μg/mL) = (1000*A470) - (1.90*Chl *a*) - (63.14*Chl *b*) (4)

Where A660, A645, and A470 represent absorbance at 660 nm, 645 nm, and 470 nm, respectively. Here,chlorophyll *a* and *b* concentrations were determined, as they interfere with the carotenoid reading at 470 nm. Determining the chlorophyll a and b concentrations subtract these values from the final carotenoid reading to get an accurate carotenoid concentration (Singh *et al*., 2019a).

Qualitative test for carotenoids:

The solvent for Thin Layer Chromatography (TLC) was made by combining acetone, benzene, and hexane in the ratio of 10:7:1. 30 ml of sample was centrifuged at 8000 rpm for 10 minutes. The obtained pellets were crushed with mortar and pestle using acetone as the solvent. The crushed sample was centrifuged at 8000 rpm for ten minutes, and the supernatant was stored at 50° C until the sample shrunk to half of its initial volume. TLC was performed using 30μl of the sample. The Rf values of the bands were calculated and the type of carotenoid was determined from the standard Rf values (Minyuk and Solovchenko, 2018).

Optimization of physical parameters:

The parameters pH and salinity were varied to find the optimal microalgae growth. Salinity is expressed as the (w/v) concentration of NaCl in media expressed in percentage. Varying salt concentrations (0%, 0.5%, 1%, and 1.5%) were used and the optimal growth was determined for a period of 7 days. To find the optimal growth at varying pH, the media was adjusted to various pH values (7, 7.5, 8, 8.5, and 9) and was added to different culture bottles, and the algae sample was inoculated. The growth was observed for a span of seven days and the maximum growth rate observed for the algae was noted. The protein and carotenoid contents were measured on the seventh day.

RESULTS AND DISCUSSION

One of the biggest threats of the twenty-first century is food insecurity, which is worsened as population growth and climate change put further strain on our already overburdened food production systems (Gordon-Strachan *et al*., 2025; Howe *et al*., 2025). New, nutritious food sources with a low ecological foot-

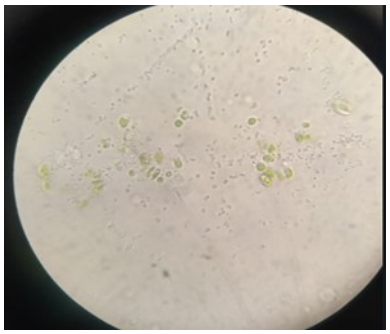

Fig. 3. *Microscopic image of Ochromonas cf. gloeopara at 100x*

print must be developed to offset the negative effects of current agricultural practices. Many of these problems can be solved by utilizing algae as an inevitable food source (Diaz *et al*., 2023; Mangena, 2024). Microalgae are considered a reliable source of vitamins, proteins, carbohydrates, and substances that are good for health, such asarotenoids (Khanashyam *et al*., 2025; Ślusarczyk *et al*., 2021). Microalgal strains have been gaining popularity in response to the industrial demand for natural substitutes, due to their ability to produce unique carotenoids under stress, such as lutein, β-carotene, α-carotene, violaxanthin, neoxanthin, and others (Sharma *et al*., 2024)

Isolation and screening of microalgae

Isolated Microalgae showed circular greenish cells of two different sizes when inoculated in BG-11 broth media (Fig. 1). The sequencing results of this sample indicated the presence of *Poterioochromonas malhamensis* (Fig. 2), a predator species that feeds on microalgae (Ma *et al*., 2018). By using 20nM phosphate the growth of predator Species was retarded (Psachoulia *et al*., 2024) and resulted in the isolation of only one type of green cells (Fig. 3). Molecular sequencing of these isolated microalgae confirmed *Ochromonas cf. gloeopara* strain CCMP2718 (Fig. 4).

Growth Kinetic Study

The standard *C. vulgaris* exhibited a maximum growth on 3rd day (16.724 h^{-1}), whereas the isolate *O. gloeopara* had a lower growth rate (15.594 h^{-1}). The growth rate of the isolate was marginally better on the 5th day where it exhibited a maximum growth rate (15.599 h^{-1}) as compared to the standard *C. vulgaris* (14.319 h^{-1}). However, *O. gloeopara* when compared to *C. vulgaris* showed a uniform growth rate throughout observation (Fig. 5). Additionally, *O. gloeopara* had a higher biomass yield of 2.2 g/l (Fig. 6) in comparison to *C. vulgaris* with a yield of 2.1 g/l after an incubation period of 7 days. The higher yield and uniform growth rate indicate that *O. gloeopara* can be cultured for periods greater than 7 days to increase the biomass. Despite the vast array of microalgae, only Haematococcus pluvialis and

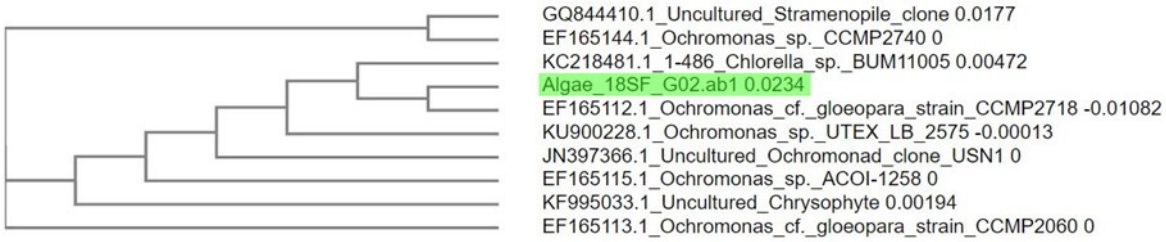

Fig. 4. *Phylogenetic tree showing the relationship of Ochromonas cf. gloeopara with closely related taxa based on partial 18S rRNA gene sequence*

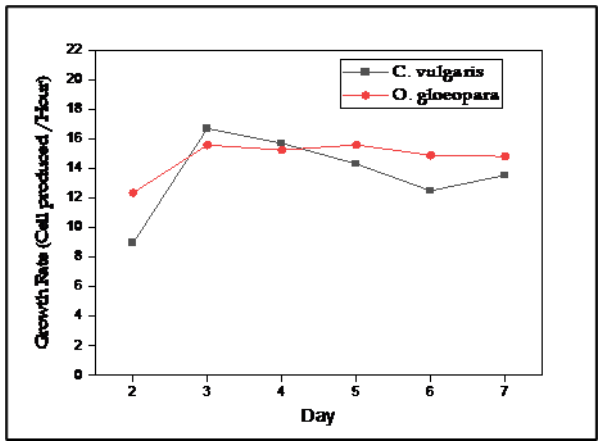

Fig. 5. *Growth rate exhibited by the Chlorella vulgaris and Ochromonas cf. gloeopara*

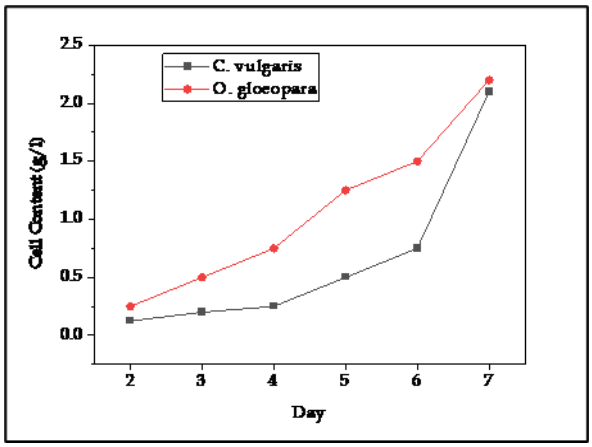

Fig. 6. *Microalgal biomass yield of Chlorella vulgaris and Ochromonas cf. gloeopara*

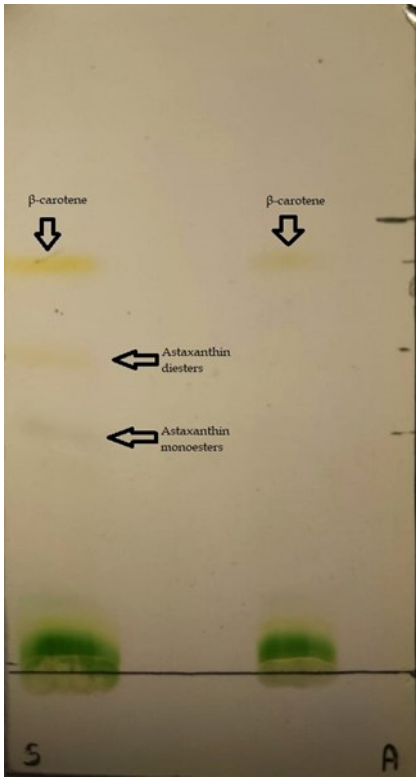

Fig. 7. *Carotenoids observed on the TLC plate (Lane S: Chlorella vulgaris and Lane A: Ochromonas cf. gloeopara)*

Dunaliella salina are commercially used for their carotenoids. However, these microalgae need particular conditions to grow and produce carotenoids(Singh *et al*., 2019)

Protein and carotenoid production

The protein content of *C. vulgaris* was found to be 24.663 mg/g and the *O. gloeopara* had 32.837 mg/g (Fig.14) of proteins. The protein yield from *O. gloeopara* was higher than Chlorella, but was similar to other microalgal species reported(El-Naggar *et al*., 2020). However, the carotenoid content in *C. vulgaris* and *O. gloeopara* was found to be 263.87µg/g and 424.645µg/g (Fig.15) which surpasses the levels of carotenoids reported in other microalgae(Yadav *et al*., 2020). These results show that *O.gloeopara* has higher protein and carotenoid content than *C. vulgaris.* Carotenoids from plants and animals have their limitations because of availability and consistency in production throughout the year. Chemical production of carotenoids is hazardous to human health because of the leftover intermediates and by-products (Senesse *et al*., 2005). Mixotrophic and photoautotrophic production of Chlorochromonas *danica* (Ochromonadales) and *Hibberdia magna* (Hibberdiales) showed 4.5-fold productivity of fucoxanthin by *C. danica*, showing that the presence of light enhanced the content compared with darkness. (Střížek *et al*., 2024).

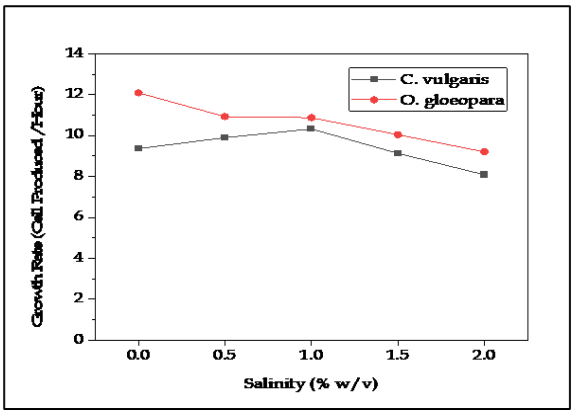

Fig. 8. *Effect of salinity on the growth rate of Chlorella vulgaris and Ochromonas cf. gloeopara*

To meet the growing demand for carotenoids, it is imperative to identify alternate sources. Thus the microalgae isolated in the present study have the potential to produce new human nutrition products because of their higher protein and carotenoid content. Carotenoids with anti-oxidant capabilities may be able to treat illnesses brought on by radicals(Adedoyin and Schmidt, 2023).In addition, microalgae are a renewable supplier of high-value, naturally occurring bioactive compounds(Cheng

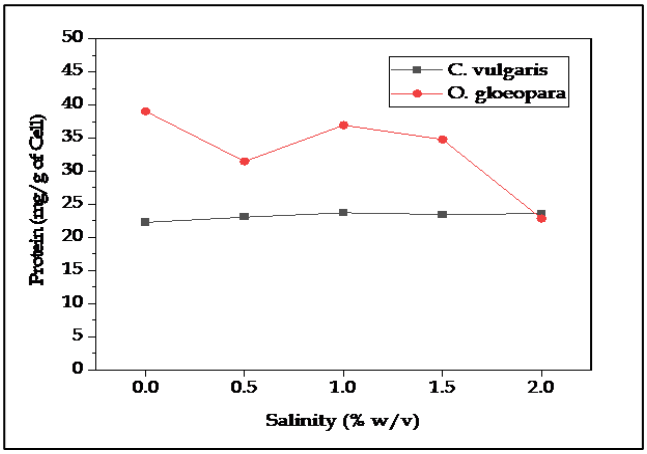

Fig. 9. *Effect of salinity on the protein content in dry biomass of Chlorella vulgaris and Ochromonas cf. gloeopara*

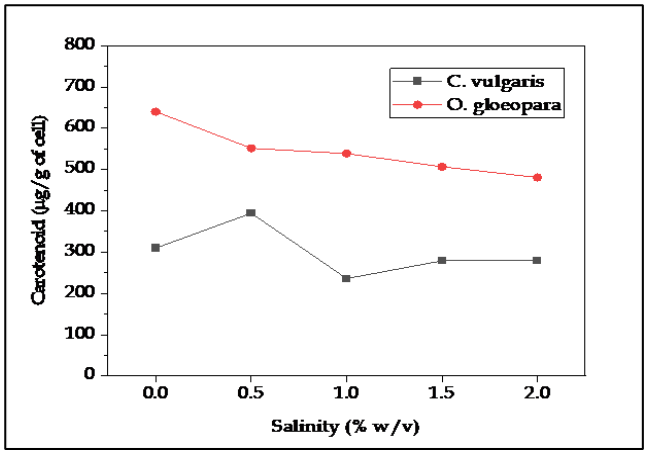

Fig. 10. *Effect of salinity on the carotenoid content in dry biomass of Chlorella vulgaris and Ochromonas cf. gloeopara*

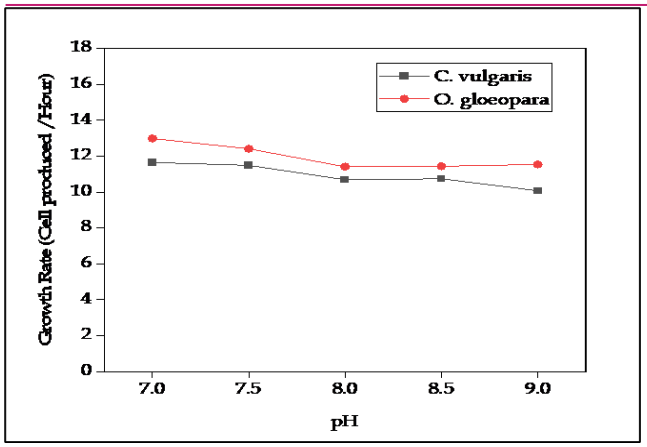

Fig. 11. *Effect of pH on the growth rate of Chlorella vulgaris and Ochromonas cf. gloeopara*

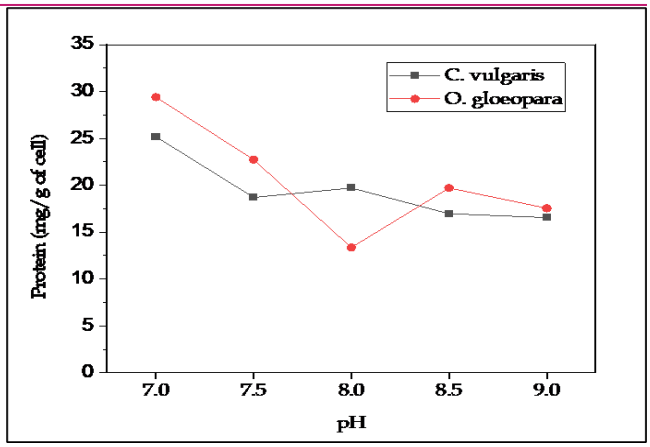

Fig. 12. *Effect of pH on the protein content in dry biomass of Chlorella vulgaris and Ochromonas cf. gloeopara*

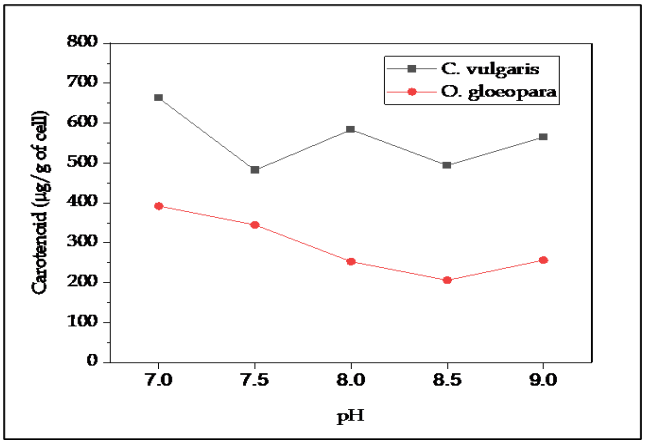

Fig. 13. *Effect of pH on the carotenoid content in dry biomass of Chlorella vulgaris and O. gloeopara*

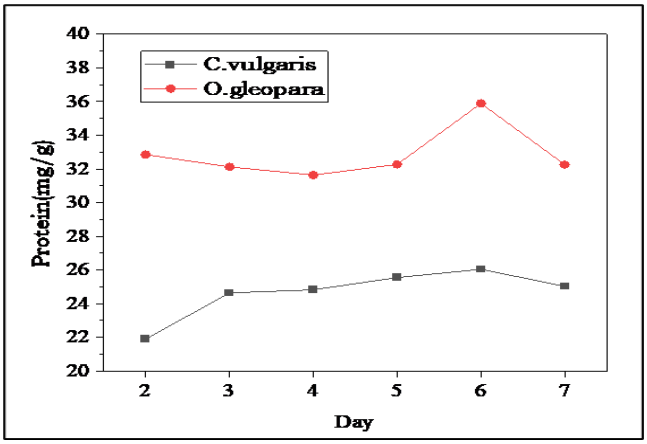

Fig. 14. *Protein content in dry biomass of Chlorella vulgaris and Ochromonas cf. gloeopara*

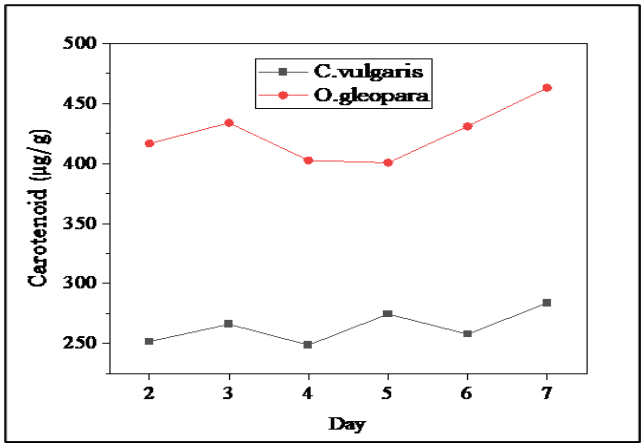

Fig. 15.*Carotenoid content in dry biomass of Chlorella vulgaris and Ochromonas cf. gloeopara*

et al., 2017; Rumin *et al*., 2021).

Qualitative test for carotenoids

Based on the repository of R_f values (Minyuk and Solovchenko, 2018) and their corresponding carotenoid type, it was found that the *C. vulgaris* produced three different carotenoid types i.e. β-carotene, Astaxanthin diesters, and Astaxanthin monoesters; whereas the *O. gloeopara* produced only β-carotene (Fig. 7). In earlier reports, *Haematococcus* sp. AA3 strain AA3 cell extracts were found to contain astaxanthin and β-carotene by TLC analysis (Adedoyin and Schmidt, 2023).Because of their high capacity to accumulate carotenoids under particular stress conditions, microalgae are particularly recognized as an effective cell factory for the production of carotenoids. 93% of chlorella is lutein, 2.6% is α- and β-carotene, and 1.3% is zeaxanthin. The minor components of chlorella are lutein, astaxanthin, β-cryptoxanthin, and other carotenoids, but β-carotene accounts for 80% of the total carotenoids produced by Arthrospira (Sharma *et al*., 2024).

Optimization of physical parameters:

Salinity optimization

The *O. gloeopara* sample showed a maximum growth rate of 12.0909 at 0% salinity (Fig. 8) when compared to the *C. vulgaris* which showed a growth rate of 10.3226 at 1% salinity. It can also be observed that *O. gloeopara* exhibited a better growth rate than *C. vulgaris* at all levels of salinity. *O. gloeopara* has the highest protein content of 39.0576 (Fig. 9) at a 0% salinity rate, whereas the *C. vulgaris* sample produced its highest protein content of 23.7266 at a 1% salinity rate. It can also be said that the salinity parameters do not affect the protein production rate of *C. vulgaris* as much as

that of *O. gloeopara.*

C. vulgaris was found to produce carotenoids of 394.3593(µg/g) at a saline concentration of 0.5% (Fig. 10), while *O. gloeopara* exhibited the highest amount of Carotenoid (640.168 µg/g) at 0% saline concentration. The *C. vulgaris* exhibited an optimum growth rate and carotenoid production in saline conditions of 1% and 0.50% respectively. Nonetheless, the production of proteins was unaffected by the salinity of the medium. In contrast, *O. gloeopara* demonstrated optimal protein production, carotenoids, and growth rate at 0% salinity.

pH optimization

The *O. gloeopara* sample exhibits a higher growth rate than the *C. vulgaris* sample. At a pH of 7, *O. gloeopara* and *C. vulgaris* showed maximum growth rates (Fig. 11) of 12.984 h^{-1} and 11.649 h^{-1}. Additionally, it can be seen that both species' growth rates decline as pH rises. *O. gloeopara* had a maximum protein content of 29.404 mg/g (Fig. 12) dry biomass at a pH of 7 and *C. vulgaris* had a maximum protein content of 28.186 mg/g dry biomass at a pH of 7. It can also be observed that as the pH increases the protein content decreases for both the species and *O. gloeopara* decreases drastically when compared to *C. vulgaris.*

Chlorella vulgaris shows higher carotenoid content than the *O. gloeopara* sample. *O. gloeopara* had a maximum carotenoid content of 391.967 µg/g (Fig. 13) dry biomass at a pH of 7 and *C. vulgaris* had a maximum protein content of 662.795 µg/g dry biomass at a pH of 7. It can also be observed that as the pH increases, the carotenoid content decreases drastically in *O. gloeopara* compared to *C. vulgaris.*

Conclusion

The study demonstrated that *O. gloeopara* can be used as a source for the production of protein and carotenoids. The *O. gloeopara* sample had a higher protein content of 32.837 mg/g when compared to *Chlorella vulgaris,* which had a protein content of 24.664 mg/g. The *O. gloeopara* also had a better carotenoid production of 424.645 µg/g than *Chlorella vulgaris* with 263.872 µg/g. The *O. gloeopara* could only produce β-carotene whereas *Chlorella vulgaris* produced β-carotene, Astaxanthin mono and diesters. *Chlorella vulgaris* had optimum growth at 1% salinity but better carotenoid production at 0.5% salinity and the salinity of the media did not affect the protein content on the other hand the *O. gloeopara* has better growth and production of Carotenoids and protein in the absence of NaCl. In media with neutral pH, both the *O. gloeopara* sample and *Chlorella vulgaris* showed enhanced growth, carotenoid, and protein production. Comparing the isolate's growth rate to that of the standard *C.vulgaris* (14.319 h^{-1}) on the fifth day showed a slightly higher maximum growth rate (15.599h^{-1}). *O. gloeopara* exhibited a constant growth rate throughout the observation period in contrast to *C. vulgaris*. Furthermore, during a 7-day incubation period*, O. gloeopara* produced a greater biomass production of 2.2 g/l compared to *C. vulgaris*. This indicates the increased yield and consistent growth rate of *O. gloeopara.* Therefore, *O. gloeopara* is a good candidate for producing protein and carotenoids, particularly β-carotene, which can be explored for production on a large scale.

Conflict of interest

The authors declare that they have no conflict of interests.

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