Bioprospecting microalgae harnessed from the coastal belt of Mangalore, India as prospective nutraceutical and biofuel candidates

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ABSTRACT
The nature of global dietary supplements is transitioning from animal derivatives to plant based. Industries around the world are seeking vegan alternatives to animal-derived supplements. The microalgal industry is expected to undergo a market boom in the current decade. Microalgal supplements (power based and extracts) as well as microalgal fuels are expected to show a significant increase in market value. Microalgae are an under-utilized source of high-value products. They are rich reservoirs of multiple compounds capable of enhancing immunity, fortifying nutritional diets and improving cardiovascular health. Microalgae are also high lipid producing organisms, which make them viable candidates in the global search for energy alternatives. However, the inability to translate in vitro results onto the industrial scale is a major drawback of the microalgal research landscape. It is therefore, vital to find a robust microalgal species capable of producing a feasible quantity of high-value compounds. This paper investigates the potential of four microalgae sourced from the coastal town of Mangalore, India as candidates for nutraceutical industries and the clean energy industry. *Desmodesmus komareikii* (MK990101) was found to be rich in total lipid, while the three *Chlorella* species were found to be relatively richer in total protein yields. *Chlorella thermophila* (MN006612) is capable of yielding harvests up to nearly 280 tonnes/acre/year. *Chlorella thermophila* (MN006612) yielded the highest amount of both mono and polyunsaturated fatty acids. The fatty acid profiles of *Chlorella thermophila* (MN006612), *Chlorella vulgaris* (MN252517-18) and *Chlorella sorokiniana* (MN011568) can be explored as an alternative edible oil. *Desmodesmus komareikii* (MK990101) yielded the highest amount of saturated fatty acids, which are used in the production of clean and quality biofuels.

Highlights

- *Desmodesmus komareikii* (MK990101) has the highest essential amino acid to non-essential amino acid ratio. However, it also has the lowest protein content, with respect to the other three species under investigation.
- The fatty acid profile of *Chlorella thermophila* (MN006612) is the rich with unsaturated fatty acids and can be explored as a candidate for edible oil production.
- The potential of *Chlorella sorokiniana* (MN011568) in the production of biodiesel favourable fatty acids makes it an interesting option for the biofuel industry.

Introduction

Microalgae and their components have been used in multiple industrial applications for decades. Their diverse nutritive characteristics combined with their ability to grow rapidly make them a viable option as dietary supplements (Paniagua-Michel, 2015). It is expected that the microalgal market (biomass or algal-based products) could generate up to $76.68 million USD by 2028 with a compounded annual growth rate of 6.9% (Data Bridge Market Research, 2021). Microalgae are notably rich in saturated fatty acids, unsaturated fatty acids and proteins. Their rich biochemistry showcases an immense potential as immune enhancing supplements (Bishop & Zubeck, 2012). A lot of biochemical analyses of microalgae have unveiled a dynamic array of metabolites that include omega-3 fatty acids, sulphated polysaccharides and carotenoids, which have anti-inflammatory/viral/bacterial properties (Barkia, Saari, & Manning, 2019). Algal proteins have been found to be helpful as broad spectrum anti-viral compounds, inhibiting viral entry and replication in cells (Ahmadi, Moghadamtousi, Abubakar, & Zandi, 2015). Their extracts also help in promoting cardiovascular health by lowering cholesterol (Reyna-Martinez...
et al., 2018; Ryu et al., 2014). Microalgal derived peptides have been used to derive Angiotensin Converting Enzyme (ACE) inhibitory peptides, which helps to lower blood pressure (Sheih, Fang, & Wu, 2009). Approximately 40–60% of the microalgal body weight (species dependent) is comprised of protein (Shim et al., 2008). Microalgal proteins can compete both qualitatively and quantitatively with the conventional sources. The market ready Chlorella based products contain high quantities of all the essential amino acids, while most commercially available non-algal protein sources tend to be deficient in a few (Williams & Laurens, 2010). Bioactive peptides from Chlorella pyrenoidosa, Nannochloropsis oculata, Arthrospira maxima and Tetraselmis suecica have proven to show anti-oxidant/inflammatory/tumour properties and also reduce hypertension (Wang & Zhang, 2013). Arthrospira is sold globally with sole emphasis on its high-quality protein content. Microalgal commercial cultivation for nutraceuticals began about 60 years ago in Japan and Taiwan with Chlorella vulgaris being the highest mass-produced chlorophycean microalgae (Beetul et al., 2016). Eventually, Japan, the United States, Israel, Australia and China branched out into the mass production of Hematococcus pluvialis and Spirulina. As per reports given by Barkia et al. (2019), Taiwan remains the highest producer of single-cell protein (SCP) products of Chlorella, producing over 2000 tons/year of the whole dried microalgae.

The unsaturated fatty acid profiles of microalgae are rich with ω3 and ω6 fatty acids (Simoons, 2014), both of which are known to promote cardiovascular health (Sayanova & Napier, 2004). Lipids are also crucial to the structural integrity of a cell and as energy reservoirs (Qu, Ren, & Huang, 2013). PUFAs have been investigated for their role in reducing inflammation, a major causative factor of Alzheimer’s (Talero et al., 2015). Several microalgae (Cryptothecodinium, Schizochytrium, Ulkenia) are cultivated for docosahexaenoic acid (DHA) which is incorporated in infant formula (Chu, 2012; Horrocks & Yeo, 1999). This product alone has an estimated market demand of $9 billion USD year⁻¹ (Martek Biosciences Corporation Annual Report, 2007). Docosapentanoic acid (DPA) has also been reported to inhibit the synthesis of the tumour necrosis factor alpha (TNF-α) (Nauroth et al., 2010). Thus, microalgae would make impressive candidates for the nutraceutical market (Paniagua-Michel, 2015). Polyunsaturated fatty acids are currently valued at $140 USD kg⁻¹ (Borowitza, 2013). Their diverse protein and fatty acid profiles also make them an impressive candidate as feed for aquacultured species. It is well known that fish oils are recommended sources of ω3/ω6 fatty acids. However, fishes generally derive their lipid constitution from microalgal diets (Medina, Grima, Giménez, & González, 1998). Additionally, microalgal oils have lower levels of contamination relative to fish oils (Gerber, Karimi, & Fitzgerald, 2012; Ryckebosch et al., 2014). Microalgal-derived feed is also utilized to increase pigmentation in shellfish (Kumar, Deviram, Mathimani, Duc, & Pugazhendhi, 2019).

The rapid depletion of energy resources has led to the seeking out of alternatives like biofuels (Kumar et al., 2019). Thus, first-generation biofuels from lipid-heavy plant crops and second-generation biofuels from edible oil were developed. However, the production of fuels from edible oil is economically disadvantageous owing to the fact that edible oil is required to meet 60% of the food requirement in India (Demirbas, 2007). Furthermore, both first- and second-generation biofuels require a lot of land and time to reach effective yield (Kumar et al., 2019). The solution to this problem lies in microalgae, resulting in third generation algal-derived fuels (Teri Energy Data Directory and Yearbook, 2011). Incidentally, the saturated fatty acid profiles of microalgae can be considered instrumental in the development of biofuels. Biofuels generated from saturated fatty acids have superior density and viscosity as well as calorific value (Talebi et al., 2013). Palmitic acid, stearic acid and lauric acid are valuable for biodiesel production (Rohit & Venkata Mohan, 2018). Generation of algal biomass does not require large allocations of land and resources, as compared to traditional fuel crops (Koyande et al., 2019). Considering that most microalgae can be grown alongside or as part of waste water systems, it creates an added advantage of combining bioremediation of water reservoirs with biomass development, thereby considerably reducing the overall cost of production (Yen et al., 2013).

A common issue with microalgal studies is the inability to translate in vitro results onto the outdoor mass-scale scenario. Although a lot of research is being driven into the development of microalgal derivatives, it is important to find a robust microalgal species capable of producing a feasible quantity of high-value compounds (Steinrücken, Erga, Mjøs, Kléivdal, & Prestegard, 2017). An algal candidate with great lipid and protein productivity might have low biomass productivity and vice versa (Mandotra, Kumar, Suseela, Nayaka, & Ramteke, 2016). Biomass productivity, therefore, becomes a crucial characteristic for the selection of high output species. High biomass productivity favours scale-up processes by reducing the energy and cost of biomass processing (Fozer et al., 2019). Many countries are foraying into this line of research voraciously. The
Indian government has pledged $87 million USD to develop algal resources of the country (Ferrer, 2021), while the Saudi government has joined hands with an Indian company, Zaara, investing $10 million USD for the development of microalgal-based food products (Mint, 2021). Indigenous microalgae display a natural tendency to adapt to changing environmental conditions (Rizza, Smachetti, Do Nascimento, Salerno, & Curatti, 2017). Identification and specific utilization of robust microalgal strains is a key step in targeted production of high-value compounds. Bioprospecting native naturally available microalgae is therefore a crucial investigative process in order to exploit them in the relative sectors of production (Matos et al., 2016). Mangalore is a coastal city with an abundance of marine, brackish and freshwater resources. It experiences a wide variation in temperatures ranging from cooler monsoons to scorching summers. The seasonal variations result in a constant intermixing of the water bodies, thereby causing a constant fluctuation in the aquatic temperature, salinity and pH, amongst other factors. It can therefore be logically presumed that the aquatic life, specifically microalgae would be capable of resisting and thereby adapting to a wide range of culture condition variations by altering their biochemistry. The present study seeks to isolate robust indigenous microalgae found in the water reservoirs of Mangalore, India and explore the possibility of utilizing the native strains as prospective microalgal industrial candidates. The investigation is based on the premise that microalgal strains found in a system that experiences frequent changes in weather pattern would be better capacious to adapt and thrive by modulating their biochemical parameters. This study seeks to bioprospect robust microalgal strains capable of naturally yielding high volumes of high-value compounds.

**Materials and methods**

**Isolation and cultivation of microalgal strains**

Freshwater samples were collected from different water reservoirs across Mangalore, Karnataka, India. The physico-chemical properties of the water samples were measured in situ using the multi-parameter PCSTestr™ 35 (Eutech instruments, Oaklon, Singapore). Various microalgae were isolated from the upper littoral zone (within 10 cm) of freshwater sources across Mangalore, Karnataka, India (Makandar & Bhatnagar, 2010). The isolates were streaked onto BG11 (Modified) agar plates (Andersen, 2005). The pH of the culture media was set to 7.4 and kept constant for all cultures. Colonies were isolated using the quadrant streak method. This was done repeatedly to isolate axenic cultures. The axenic cultures were grown at 27 ± 1°C, 16: 8 light: dark cycle at 20–24 μmol m−2 s−1 PPFD/1600 lux using fluorescent white tubes (to reduce the probability of photoinhibition). Growth was analysed periodically at a five-day interval. It was estimated as a measure of the optical density at 600 nm (Mandotra et al., 2016). Biomass productivity was assessed as per Mandotra et al. (2016):

\[
BP = \frac{(B2 - B1)}{(T2 - T1)}
\]

Where BP is the biomass productivity (mg l−1 day−1), B2 and B1 are the biomass harvested (mg l−1) at two sampling times T2 and T1 respectively.

The data obtained for biomass were extrapolated to calculate the possible harvest outcomes in terms of tonnes acre−1 year−1. The biomass extrapolation was performed by considering an open raceway pond as a means of biomass generation. The extrapolation is based on the existing pond cultures employed in our laboratory’s investigations, in order to ensure a realistic extrapolation. Our study employed open raceway ponds of 15 ft² area bearing the volume capacity of 500 l. The extrapolation was performed considering the use of the open ponds for the parameters mentioned against a land area of one acre.

For the purpose of this paper, the first four isolates showing maximum growth are discussed. Algal cells were harvested on the 45th day post-inoculation to ensure sufficient biomass volume by centrifuging at 8534 G (10 min, 25°C) and subjected to further testing. The wet weights and dry weights of the algal biomass were determined gravimetrically. The algal pellet was subsequently dried at 40°C for 36 h, following which the biomass was ground and sieved through a 45 μm mesh to obtain uniformly sized algal powder. The powder was transferred to vials flushed with nitrogen gas, sealed and stored at –20°C for further analysis.

**Strain identification (Fawley & Fawley, 2020)**

For strain identification, three-week-old axenic strains were selected for morphological analyses. The light microscopic investigation was done by suspending the strain in 1% Lugol’s Iodine solution for 24 h, with subsequent photographic documentation using Olympus BX-41 (Japan) light microscope with an attached CCD camera (Olympus DP 73, Japan). Axenic cells were harvested by centrifugation and broken into a bead mill. DNA was extracted using the HI Media Hi-Pur A Marine Algae DNA purification kit. The 18S rDNA sequence was amplified and sequencing of molecular data was outsourced to Eurofins Scientific, Bangalore, India. Forward and reverse DNA sequencing reaction of PCR
amplicon was carried out with NS1 and NS4 primers bearing sequences GTAAGCATATGGCTTGCCTC and CTTCCGTCAATTCTTTTAAG respectively using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyser (Thermo Fischer Scientific, Japan). Sequence data was cross-referenced using BLAST against available data sequences in the NCBI GenBank Database. The first 10 sequences with maximum identity scores were selected and aligned using ClustalW. A phylogenetic tree was constructed using MEGA7. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura-2 parameter model (Kimura, 1980). The strain sequence data was registered and deposited with GenBank.

**Total lipid and fatty acid analyses**

For lipid analyses, 2 g of the algal biomass was resuspended and washed thrice with distilled water. The washed pellets were oven dried at 40°C, overnight. The dry cell biomass was subjected to the modified Bligh and Dyer (1959) method using chloroform and methanol (3:2) as per Ming et al. (2012). To maximize lipid yield, extraction was repeated thrice. To the pooled extract, 1% sodium chloride and sodium sulphate crystals were added to remove any water-soluble impurities and moisture. The lipid extracts were dried under nitrogen gas and the weight of the lipids were determined gravimetrically (Prabharan & Uma, 2017).

For fatty acid analysis (Prabharan & Uma, 2017), the lipid extracts were refluxed with 1% v/v sulphuric acid in methanol at 70°C for two hours. The esterified contents were transferred to a centrifuge tube and equal volumes of distilled water and hexane were added. The upper layer containing the fatty acid methylated esters (FAMEs) were collected and dried under nitrogen gas following which gas chromatographic analyses were carried out (Feng, Chen, Xue, & Zhang, 2011). 2 µl of the lipid samples were injected into the gas chromatograph (Agilent/7890B). The fatty acid profile was measured using gas chromatograph-flame ionization detector (GC-FID) have a DB-23 Agilent column (60 mm × 0.32 mm, 0.25 µm thick). The temperature of both the injector and detector were maintained at 250°C (Hena, Fatimah, & Tabassum, 2015). The oven temperature was initially maintained at 50°C for three minutes, followed by an incremental increase of 10°C to the final temperature of 130°C for one minute. The oven temperature was increased incrementally (4.5°C) to a final temperature of 245°C for a total of seven minutes. Nitrogen gas was used as the carrier gas, maintaining the flow rate at 1 ml min⁻¹. Supelco CRM47885 was used as the standard FAME mix to calculate the retention time of each FAME.

**Statistical analyses**

All experimental setups were maintained in triplicates. The data were analysed and plotted using MS Excel Professional Plus (2019). To elucidate the relationships between the microalgal strains, all data values were subjected to ANOVA.

**Results and discussion**

**Strain identification and cultivation**

Our study allowed us to isolate and identify four chlorophycean microalgal species. The sequence data of each
species was deposited into GenBank and an accession code was allocated (Table 1). Microalgal growth occurs relative to the interactions of physical and chemical parameters. Elevated parameters support the inclination of the water body towards eutrophication (Naik, Vinod, & Kusuma, 2010). Physico-chemical analyses of the sampling site parameters showed that while all three Chlorella species grew in moderate (pH 7.3–7.5) to highly alkaline waters (pH 9.35), D. komareikii (MK990101) grew in mildly acidic waters. Alkaline pH usually signifies high photosynthetic activity pointing to a probability of eutrophication of the water bodies (Naik et al., 2010). Salinity and total dissolved solids (TDS) are inversely proportional to the level of dissolved oxygen in the water bodies (Shrivastava, Bharadwaj, & Shrivastava, 2014). Table 1 indicates that all four microalgal strains were isolated from water bodies having low levels of salinity and TDS. This indicates a high level of dissolved oxygen. Eutrophied water bodies display high levels of dissolved oxygen during the day, on account of the high photosynthetic activity of algal blooms (Horrigan, Lawrence, & Walker, 2002). Considering that all algal sampling of this study was performed during daylight, it would be safe to assume that at the time of sampling, the microalgae were isolated from eutrophied water bodies.

**Table 1.** Location parameters defining the natural growth conditions of isolated microalgae. Identified microalgae were allocated GenBank accession numbers as defined in column 2.

| Organism               | Gen bank accession code | Location of sampling      | GPS coordinates | Location description          |
|------------------------|-------------------------|---------------------------|-----------------|-----------------------------|
| Chlorella thermophila  | MN006612                | Surathkal lighthouse, Dakshina Kannada | 13.0060° N | pH: 9.35 Conductivity: 116.2 μS TDS: 81.6 ppm Salinity: 159 ppm |
| Chlorella vulgaris      | MN252517-18             | Kannada, Falnir, Mangalore | 27.2046°N | pH: 7.3 Conductivity: 72.1 μS TDS: 52.3 ppm Salinity: 40.2 ppm |
| Chlorella sorokiniana  | MN011568                | Hosabettu, Mangalore      | 13.0632°N | pH: 7.98 Conductivity: 116.2 μS TDS: 81.6 Salinity: 235 ppm |
| Desmodesmus komareikii | MK990101                | Iddya, Surathkal          | 12.9824°N | pH: 6 Conductivity: 113.6 μS TDS: 80.7 ppm Salinity: 59 ppm |

**Chlorella thermophila (MN006612)**

This species was isolated from a freshwater pond near the Surathkal lighthouse, Mangalore overlooking the Arabian sea. Investigation of the axenic cultures by bright field microscopy revealed unicellular solitary spherical chlorophyceans. The cells have cup-shaped chloroplasts with a single pyrenoid (Fig 2A). Molecular amplicon of the 18S DNA sequence gave a 1118 bp long sequence that showed high similarity with C. thermophila KF 661,334 (99.32%) (Fig 1A), against the outgroup of C. vulgaris. Sequence data was deposited in GenBank. Axenic cultures were upscaled in BG11 solid and liquid media.

**Chlorella vulgaris (MN252517-18)**

C. vulgaris is characterized by a distinct rigid cell wall housing dense green cytoplasm (Fig 2B). This particular strain was isolated from a freshwater well in Falnir, Mangalore. Morphologically, it is similar to other species described here such as C. thermophila and C. sorokiniana. However, molecular data identified the molecular amplicon (763 bp) of this strain as a relative
to 10 other strains of *C. vulgaris*, showing the highest percentage similarity with KX639565 (94.77%) (Fig 1B).

**Chlorella sorokiniana (MN011568)**

As is typical of any *Chlorella* species, *C. sorokiniana* too is a chlorophycean microalga, found as unicellular organism in water. The cells have cup shaped chloroplasts with a single pyrenoid (Fig 2C). The molecular amplicon (1124 bp) of this organism placed it close to both *C. thermophila* and *C. sorokiniana*, against the outgroup of *C. vulgaris*. However, the percentage similarity to *C. sorokiniana* (MG597606) was higher (99.71%) (Fig 1C).

**Desmodesmus komareikii (MK990101)**

*Desmodesmus* species are colonial chlorophycean microalgae usually found as multiples of two. These microalgae have the presence of a pyrenoid at either end of the oblong cell body (Fig 2D). The outer cell bodies have the presence of two thick spines running at both corners. *D. komareikii* was isolated from a freshwater pond in Iddya, Surathkal, Mangalore. The 18s DNA amplicon (1023bp) of this species showed 99.23% percentage identity with *D. komareikii* (AB818541) (Fig 1D) against the outgroup of *D. armatus*.

**Proximate analyses**

Proximate analyses of the microalgae showed that the dry weight of the identified freshwater microalgae is made up of 41.5%–26.7% protein. This corroborates the data given by Boyd (1973). Of the four microalgal species studied, *C. thermophila* (MN006612) and *D. komareikii* (MK990101) produced the highest biomass of more than 9 g l⁻¹ of the algal culture suspension. Their biomass productivity was also higher with *D. komareikii* (MK990101) showing the highest productivity at 482.4 ± 0.2 mg l⁻¹ d⁻¹. The biomass and biomass productivity of both *C. vulgaris* (MN252517-18) and *C. sorokiniana* (MN011568) were not significantly different from each other. However, all three *Chlorella*
species produced significantly higher quantities of total protein (p < 0.05) relative to *D. komarekii* (MK990101) which yielded only about 26% protein of its total biomass. Proximate analyses of the microalgae confirm the studies of Fabregas and Herrero (1985) stating that *Chlorella* species would be better suited for the production of single-cell proteins (SCPs). In contrast, the analysis of total lipids showed that *D. komarekii* (MK990101) yielded significantly higher volume of lipids (p < 0.05) with up to 178.5 mg g⁻¹ biomass. However, all three *Chlorella* species yielded lower quantities of lipid (2.3–5.5% lipids). The proximate analyses of the microalgae conclusively prove that *Chlorella* species would be better suited for protein investigation and products, while *D. komarekii* (MK990101) has potential in lipid-related ventures. The data obtained for biomass, protein and lipid was extrapolated to calculate the possible harvest outcomes in terms of tonnes acre⁻¹ year⁻¹. As represented in Table 2, *C. thermophila* (MN006612) is capable of yielding harvests up to nearly 280 tonnes acre⁻¹ year⁻¹, with estimated harvest of 2800 tonnes year⁻¹ over 10 acres. This is considerably higher than Taiwan, which currently produces the highest yields of *C. vulgaris*, of nearly 2000 tonnes year⁻¹ (Barkia et al., 2019). Due to the advancing interests in microalgal lipids, many species have been cultured on a mass scale for lipid harvests. However, *D. komarekii* (MK990101) remains an underutilized species. Table 2 suggests that *D. komarekii* (MK990101) is capable of producing nearly 37.4 ton acre⁻¹ year⁻¹ lipids.

**Amino acid analyses**

The amino acid profiles of *C. thermophila* (MN006612), *C. vulgaris* (MN255217-18), *C. sorokiniana* (MN011568) and *D. komarekii* (MK990101) are presented in Table 4. The essential amino acids (EAAs) for humans include histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. *D. komarekii* (MK990101) yielded the highest quantity (Table 2, Fig 3) of all nine EAAs followed by *C. thermophila* (MN006612) (producing more than 1000 mg each of seven EAAs) and *C. sorokiniana* (MN011568) (more than 1000 mg each of four EAAs) (Table 4).

Histidine is an important precursor in protein synthesis. It is also an important part of the active sites of enzymes, making up a part of the catalytic triad (Attia, Al-Harthi, Korish, & Shiboob, 2020; Brown, 1991). The recommended dose allowance (RDA) for histidine is 700 mg day⁻¹. *D. komarekii* (MK990101) produces up to nine times this amount while *C. thermophila* (MN006612) produced 70 mg more than the recommended dose. Isoleucine is responsible for increasing the nitrogen content of muscle cells (Derrien, Coiffard, Coiffard, & De Roeck-Holtzhauser, 1998). *D. komarekii* (MK990101) and *C. thermophila* (MN006612) produce more than 1000 mg 10⁻¹ biomas of this amino acid. Leucine plays an important role in regulating blood sugar as well as in muscle health (Attia et al., 2020). To this end, the RDA of leucine is 2730 mg day⁻¹. However, an egg supplements about 1091 mg of leucine. In contrast to this, *D. komarekii* (MK990101), *C. thermophila* (MN006612) and *C. sorokiniana* (MN011568) are able to supplement 3510 mg, 2500 mg and 1810 mg per 100 g algal biomass respectively. Lysine regulates the carnitine cycle and plays a crucial role in cholesterol regulation (Akgül, Kızılkaya, Akgül, & Erduşan, 2015). *D. komarekii* (MK990101) matches up to the RDA of lysine (p < 0.03), producing 530 mg more than recommended. Methionine is another amino acid that promotes muscle growth, creatine production and cartilage production (Nimalaratne, Lopes-Lutz, Schieber, & Wu, 2011). *C. thermophila* (MN006612), *D. komarekii* (MK990101) and *C. sorokiniana* (MN011568) produce close to 400 mg of methionine. However, all four chlorophyceans yield lesser than the RDA of 728 mg day⁻¹. Phenylalanine plays a definite role in the production of thyroid hormone and therefore is important in ensuring active metabolism (Brown, 1991). It is also crucial in the production of the adrenal hormones-epinephrine and norepinephrine.

**Table 2.** Proximate analyses of the isolated microalgae. *w* w denote significant differences between groups (p < 0.05). Groups sharing symbols are significant to each other. The table clearly illustrates that maximum biomass was produced by *D. komarekii* (MK990101) followed by *C. thermophila* (MN006612). However, the total protein content was higher in all three *Chlorella* species relative to *D. komarekii* (MK990101).

| Organism | *C. thermophila* MN006612 | *C. vulgaris* MN255217-18 | *C. sorokiniana* MN011568 | *D. komarekii* MK990101 |
|----------|----------------|----------------|----------------|----------------|
| Total Dry Biomass (g l⁻¹) | 16.7 ± 0.2* | 8.7 ± 0.2* | 8.7 ± 0.2* | 15.8 ± 0.1* |
| Biomass Productivity (mg l⁻¹ d⁻¹) | 514.4 ± 0.4* | 266.1 ± 0.8* | 266.1 ± 0.8* | 482.4 ± 0.1* |
| Biomass extrapolated (tonnes acre⁻¹ year⁻¹) | 278.8 ± 1.0* | 156.8 ± 1.2* | 156.8 ± 1.2* | 209.1 ± 1.0* |
| Total Protein Content (mg g⁻¹) | 415.3 ± 0.6* | 322.3 ± 0.6 | 274.8 ± 0.3* | 266.7 ± 0.6* |
| Protein extrapolated (tonnes acre⁻¹ year⁻¹) | 120.8 ± 0.2* | 48.8 ± 0.3* | 48.8 ± 0.3* | 73.4 ± 0.3* |
| Total Lipid Content (mg g⁻¹) | 55 ± 0.2* | 46 ± 0.5* | 22.5 ± 0.8* | 178.5 ± 0.8* |
| Lipid extrapolated (tonnes acre⁻¹ year⁻¹) | 16 ± 0.1* | 6.9 ± 0.3* | 3.4 ± 0.1* | 49.2 ± 0.4* |
All four microalgal species (p < 0.005) produced more than 1300 mg of phenylalanine, nearly twice as much as found in an average egg. The same can be said about threonine and valine production, both of which are essential in maintaining healthy brain and muscle chemistry (Attia et al., 2020). Tyrosine, a conditional non-essential amino acid (Whitney & Rolfs, 2018) is synthesized from phenylalanine. However, certain genetic conditions can block this synthesis process, thereby rendering it an essential amino acid in such patients. *D. komarekii* (MK990101) yielded the highest tyrosine (p < 0.05) of the four microalgae (1150 mg 100 g⁻¹) (Table 3). In comparison with the regularly recommended supplement of an egg, it can be seen that all four microalgae produced considerably higher amounts of all the EAAs. An exception to this was *C. vulgaris* (MN252517-18) which generated 87.7 mg and 83.8 mg per 100 mg biomass lesser isoleucine and methionine respectively. Additionally, *C. sorokiniana* (MN011568) also produces lesser lysine in comparison to an egg. As per Attia et al. (2020), an egg of about 60 g provides roughly 17.4–26.7% of the total recommended dietary allowance (RDA) of protein. Therefore, in order to fulfil a person’s complete RDA requirements, one would have to consume three to four eggs, approximately 240 g. In contrast to this, *C. thermophila* (MN006612) fulfils 82.2% RDA 100 g⁻¹ biomass, *C. vulgaris* (MN252517-18) provides 51.1% RDA/100 g biomass, *C. sorokiniana* (MN011568) fulfils 65.5% RDA 100 g⁻¹ biomass and *D. komarekii* (MK990101) provides 156.5% RDA 100 g⁻¹ biomass. On an average, approximately 200 g of any of the microalgae would satisfy the protein dietary requirements of a person.

**Fatty acid analyses**

Microalgal lipids generally accumulate when the organisms are under stress (Yen et al., 2013). FAME analysis of the four microalgae revealed a range of saturated fatty acids (SFAs), poly/monounsaturated fatty acids (PUFAs/MUFAs). All microalgae yielded close to 50% SFAs of the total lipid content (Table 4). The primary SFAs detected in high quantities were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and behenic acid (C22:0). PUFAs constituted nearly 35% of the total lipid content in three of the four microalgae. However, *D. komarekii* (MK990101) had the lowest fraction of PUFAs comprising of 18.91% of the total lipid fraction. Three PUFAs were detected of which linoleic acid (C18:2) and linolenic acid (C18:3) were of considerably higher quantities (Fig 4). The MUFAs profile varied between the four microalgae, being highest in *C. thermophila* (MN006612) (12.2%) and lowest in *C. sorokiniana* (MN011568) (7.9%).

**Saturated fatty acids (SFAs)**

Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and behenic acid (C22:0) were dominant across all the four microalgal species. *D. komarekii* (MK990101) yielded the highest fraction of SFAs (72.1%) followed by *C. sorokiniana* (MN011568) (57.2%), *C. vulgaris* (MN252517-18) (56.6%) and lastly *C. thermophila*.
Table 3. Amino acid profiles of the investigated microalgae against the amino acid profile of a regularly recommended protein source of an egg (Attia et al., 2020) and the recommended daily allowance (RDA) of each amino acid as given by the Food and Agriculture Organization (Attia et al., 2020). Essential amino acids are highlighted in bold.

| Amino Acids | Weight (mg 100 g⁻¹) | Weight (mg 100 g⁻¹) | Weight (mg 100 g⁻¹) | Weight (mg 100 g⁻¹) | Weight in 1 egg (mg 100 g⁻¹) | RDA (mg kg⁻¹ d⁻¹) |
|-------------|----------------------|---------------------|---------------------|---------------------|----------------------------|---------------------|
|             | C. thermophila MN006612 biomass | C. vulgaris MN252517-18 biomass | C. sorokiniana MN011568 biomass | D. komarekii MK90101 biomass | (Attia et al. 2020) | (Attia et al. 2020) |
| Alanine     | 1990                 | 1250                | 1420                | 2840                | 664.8                     | -                   |
| Arginine    | 3110                 | 1770                | 1230                | 2730                | 814.9                     | -                   |
| Aspartic acid| 2080                | 1140                | 1360                | 4070                | 1271.2                    | -                   |
| Cystein/Cystine | 300              | 250                 | 330                 | 230                 | 546.4                     | 15 (1050 mg d⁻¹)    |
| Glutamic acid| 3270                | 1390                | 2290                | 5130                | 1649.6                    | -                   |
| Glycine     | 3860                 | 2680                | 2760                | 5580                | 430.7                     | -                   |
| Histidine   | 770                  | 560                 | 630                 | 6480                | 312.3                     | 10 (700 mg d⁻¹)     |
| Isoleucine  | 1050                 | 580                 | 740                 | 1460                | 668.3                     | 20 (1400 mg d⁻¹)    |
| Leucine     | 2500                 | 1490                | 1810                | 3510                | 1091.8                    | 39 (2730 mg d⁻¹)    |
| Lysine      | 1370                 | 740                 | 940                 | 2630                | 956.3                     | 30 (2100 mg d⁻¹)    |
| Methionine  | 440                  | 270                 | 370                 | 420                 | 353.8                     | 10.4 (728 mg d⁻¹)   |
| Phenylalanine| 1770                | 1320                | 1350                | 2150                | 645.1                     | -                   |
| Proline     | 1700                 | 1060                | 1510                | 2170                | 514.9                     | -                   |
| Serine      | 1380                 | 980                 | 980                 | 1990                | 998.2                     | -                   |
| Threonine   | 1330                 | 860                 | 1490                | 1450                | 614.4                     | 15 (1050 mg d⁻¹)    |
| Tyrosine    | 1090                 | 860                 | 970                 | 1150                | 1168.4                    | 25 (1750 mg d⁻¹)    |
| Valine      | 1550                 | 910                 | 1100                | 2120                | 744.6                     | 26 (1820 mg d⁻¹)    |
| EAA: NEAA   | 0.6                  | 0.7                 | 0.8                 | 0.8                 | 0.7                       | -                   |
indicates saturation. Rohit stearic Chlorella were highest (MK990101) (C18:3, species. yield microalgal Polyunsaturated fractions. Saturated fatty acids such as palmitic and stearic acid are considered valuable for biodiesel production (Bajhaiya, Mandotra, Suseela, Toppo, & Ranade, 2010; Rohit & Venkata Mohan, 2018). The higher degree of saturation results in better density and viscosity resulting in better biodiesel (Chandra, Rohit, Swamy, & Mohan, 2014; Talebi et al., 2013). The degree of saturation also indicates a higher calorific value, a characteristic favourable to biofuels.

**Polynsaturated fatty acids (PUFAs)**

The PUFA profiles showed a marked variation across the microalgal lipid fractions. *C. thermophila* (MN006612) yielded the highest fraction of PUFAs (38%). However, the individual PUFAs varied across the four microalgal species. Linoleic acid (C18:2), Gamma linolenic acid (C18:3, n6), linolenic acid (C18:3) and cis 11,14,17 eicosatrienoic acid (C20:3) were detected. C18:2, C18:3 and C20:3 have been reported to reduce inflammation (Saini & Keum, 2018), cardiovascular diseases (Schmidt, Skou, Christensen, & Dyerberg, 2000) and also have anti-inflammatory properties (Adarime-Vega et al., 2012; López et al., 2019). Polynsaturated fatty acids are valuable in the commercial nutraceutical market due to their ability to reduce diabetes (Krishna Mohan & Das, 2001), arthritis (Paniagua-Michel, 2015) and effectively reducing heart diseases ( Howe, 1997). Gamma linolenic acid in particular has a role to play in nutraceutical production (Rohit & Venkata Mohan, 2018). PUFAs are important precursors of Ω3 and Ω6 fatty acid (Kumar et al., 2019). Linoleic acid is also a key Ω3 fatty acid while linolenic acid is a key Ω6 fatty acid. All three *Chlorella* species yielded higher amounts of both linoleic and linolenic acid in comparison to *D. komareikii* (MK990101). Amongst the *Chlorella* species, *C. vulgaris* (MN252517-18) yielded the largest quantity of linoleic acid (24.5%) while *C. thermophila* (MN006612) yielded larger quantities of linolenic acid (19.4%).

**Monounsaturated fatty acids (MUFA)**

Monounsaturated fatty acids are commonly used as edible oils. The monounsaturatoin is a characterisitc for cooking oils and can help to reduce the low-

### Table 4. Fatty acid profiles of the investigated microalgae, segregated as saturated and unsaturated fatty acids. *D. komareikii* (MK990101) yielded the highest amount of saturated fatty acids (72.1% of the total lipid yield). *C. thermophila* (MN006612) yielded highest amount of unsaturated fatty acids (38% PUFA and 12.2% MUFA).

| %SFA                        | C. thermophila (MN006612 (% distribution)) | C. vulgaris (MN252517-18 (% distribution)) | C. sorokiniana (MN011568 (% distribution)) | D. komareikii (MK990101 (% distribution)) |
|-----------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|
| Octadecenoic acid (C18:1)  | 1.2                                       | 1.9                                       | NA                                        | 0.1                                       |
| Tetradecanoic acid (C14:0) | 0.6                                       | 0.9                                       | 0.4                                       | 2.3                                       |
| Hexadecanoic acid (C16:0)  | 25.6                                      | 24.2                                      | 27.6                                      | 18.9                                      |
| Heptadecanoic acid (C17:0) | 0.4                                       | 0.5                                       | 0.6                                       | NA                                        |
| Eicosenoic acid (C20:0)    | 1.2                                       | 1.7                                       | 0.3                                       | NA                                        |
| Docosanoic acid (C22:0)    | 8.2                                       | 10.1                                      | 12.6                                      | 4.7                                       |
| %MUFA                      |                                            |                                            |                                            |                                            |
| Tetradecenoic acid (C14:1) | NA                                        | NA have (NA                                  | 0.1                                       | NA                                        |
| Hexadecanoic acid (C16:1)  | 0.5                                       | 0.6                                       | 0.6                                       | 0.4                                       |
| 9-Octadecanoic acid (C18:1)| 3.8                                       | 3.9                                       | 3.9                                       | 3.9                                       |
| %PUFA                      |                                            |                                            |                                            |                                            |
| 9,12-Octadecadienoic acid (C18:2) | 18.7                             | 24.5                                      | 19.7                                      | 10.7                                      |
| 9,12,15-Octadecatrienoic acid (C18:3) | 19.4                             | 10.2                                      | 18.9                                      | 8.2                                       |
| 9,11,14,17-Eicosatrienoic acid (C20:3) | NA                             | 0.4                                       | 0.4                                       | NA                                        |
| Saturated fat (%)          | 49.8                                      | 56.6                                      | 57.2                                      | 72.1                                      |
| PUFA (%)                   | 38.0                                      | 35.1                                      | 34.6                                      | 18.9                                      |
| MUFA (%)                   | 12.2                                      | 8.4                                       | 7.9                                       | 8.9                                       |
density lipoproteins (Teres et al., 2008). Palmitoleic acid (C16:1) and oleic acid (C18:1) have been reported to reduce blood pressure and thereby play a role in preventing heart ailments (Rohit & Venkata Mohan, 2018). The highest amount of MUFAs were detected in C. thermophila (MN006612) (12.2%). Of the five MUFAs detected, cis-10 heptadecenoic acid (C17:1) was found predominantly in the three Chlorella species. Oleic acid (C18:1) was significantly higher in D. komarekii (MK990101), constituting 5.6% of the total lipid yield. López et al., (2019) reported that C18:1 play a role in the structure of ceramides and sphingolipids. This makes C18:1 a key fatty acid in the anti-inflammatory pathway (Kapoor & Huang, 2006). Apart from this, C18:1 is also used in relieving pain, anxiety and other mental disorders such as bulimia (Whiley, Godzien, Ruperez, Legido-Quigley, & Barbas, 2012).

Conclusion

Microalgae have long been explored as viable alternatives to both energy resources as well as health supplementation. Even though the microalgal supplements have received negative attention, for containing heavy metal contaminants, the microalgal industry is expected to undergo a huge boom between 2021 and 2028 (Data Bridge Market Research, 2021). Whole microalgal biomass and its derivates are vegan alternatives to traditionally processed protein and lipid supplements. Powder or capsule forms of the microalgal supplements are expected to dominate the markets for the next decade (Data Bridge Market Research, 2021). The current market focuses on using and exploiting Spirulina and C. vulgaris for SCP production. However, most other species of Chlorella remain unharnessed on an industrial scale. Chlorella species are considered a superfood, superior to recommended animal-derived supplements (Koyande et al., 2019). This investigation looked into harnessing the local microalgae of Mangalore, India as potent industrial candidates. Of the microalgae investigated, D. komarekii (MK990101) was found to be rich in total lipid, while the three Chlorella species were found to be relatively richer in total protein yields. C. thermophila (MN006612) is capable yielding harvests up to nearly 280 tonnes acre\(^{-1}\) year\(^{-1}\). It can be noted that D. komarekii (MK990101) was considerably richer in the essential amino acids, relative to the other algal species as well as to the regularly recommended natural protein source of an egg. Therefore, D. komarekii (MK990101) has potential to be explored as a vegan protein supplement, for both human as well as aquaculture feed. Unsaturated fatty acids play a key role in maintaining cardiovascular health. All three Chlorella species showed high yields of both mono and polyunsaturated fatty acids. C. thermophila (MN006612) yielded the highest amount of both mono and polyunsaturated fatty acids. The fatty acid profiles of C. thermophila (MN006612), C. vulgaris (MN252517-18) and C. sorokiniana (MN011568) can be explored as an alternative edible oil. D. komarekii (MK990101) yielded
the highest amount of saturated fatty acids, which are used in the production of clean and quality biofuels. *D. komareikii* (MK990101) is capable of producing nearly 37.4 ton acre\(^{-1}\) year\(^{-1}\) of oil. It would be worthwhile to explore these robust strains of *D. komareikii*(MK990101) in the production of biofuels.

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**Availability of data and material**

All data and material are available on request.

**Consent for publication**

All authors approve of the manuscript for publication.

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