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Short Communication

New insights into the biochemical composition of estuarine diatom *Halamphora* sp. isolated from Munambam-Azheekode Estuary, Kerala, India

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Halamphora sp., an amphoroid diatom, collected and isolated from the Munambam-Azheekode Estuary within the Vembanad-Kol, Ramsar site of India's Kerala state. The biochemical composition of *Halamphora sp.* is depicted with special reference to salinity changes (10, 20, 30, and 40 psu) at two culture stages. The highest growth rate and maximum cell density occurred at 30 psu. Lower salinities promoted higher protein and carbohydrate content during the stationary phase, while lipid production was higher in the log phase at lower salinities. Notable amounts of short, medium, and long-chain fatty acids, with C20:5 and C22:6 being particularly prominent, along with increased levels of C18:3n3 Polyunsaturated Fatty Acids (PUFAs). The findings recommend that 30 psu provides optimal growth and nutritional composition for *Halamphora* sp. in various industrial applications, especially in the production of biodiesel and aquaculture feeds.

[**Keywords**: Estuarine diatom, Fatty acids, Microalgae culture, Proximate composition]

Introduction

Diatoms are important environmental indicators, because of their quick response to ecosystem changes[1]. Microalgae are recognised as a promising biodiesel source, due to their high photosynthetic efficiency and growth rate[2-4]. However, this requires a high amount of lipid, which would otherwise decrease economic performance[5]. Due to their high fatty acid content, diatoms are considered valuable for nutraceutical production. They are a source of important long-chain Polyunsaturated Fatty Acids (PUFAs) like Eicosapentaenoic Acid (EPA), Arachidonic Acid (ARA), and Docosahexaenoic Acid (DHA). These compounds are widely used in health supplements, food enrichment and animal feeds to improve fat profiles[6].

Environmental variables such as light, nutrients, temperature, pH and salinity influence their chemical composition[7,8], with salinity being a significant factor determining diatom distribution in estuaries. Field studies suggest that salinity (caused by tides, fluctuating freshwater inputs and rainfall) tolerance is a prerequisite for diatoms inhabiting estuaries and coastal wetlands[9-11]. Nevertheless, culture-based works are necessary to understand their role in determining their natural distribution.

It is crucial to identify the optimal salinity for culturing *Halamphora* sp., collected from the Azheekode Estuary, part of the Vembanad-Kol Ramsar site. The species was isolated and cultured axenically to study its phytochemical characteristics, which may be utilised for biodiesel and aquacultural purposes. This investigation aimed to determine the effects of different salinities on the growth and biochemical composition of the diatom during distinct growth phases in laboratory cultures.

Materials and Methods

Sample collection, isolation and identification

Samples collected at a salinity of 27 psu from the Munambam-Azheekode Estuary, part of the Vembanad-Kol Ramsar site (10°10′48.29″ N and 76°10′04.14″ E), Kerala, India, were isolated by serial dilution and agar plating. Live cells were identified by light and scanning electron microscopy and classified at the genus level using standard keys from India and abroad.

DNA and PCR amplification

Further, for molecular taxonomy, PCR amplification and sequencing were carried out using the method described by White *et al.*[12]. DNA was isolated using the NucleoSpin® Plant II Kit (Macherey-Nagel). The primers used for the process were LROR (Forward Primer: ACCCGCTGAA CTTAAGC) and LR7 (Reverse Primer: TACTACCACCAAGATCT), both from 5' to 3'.

Culture conditions

Axenic cultures were prepared using antibiotics and confirmed through nutrient agar plating. These were sub-cultured and inoculated into 2 L Erlenmeyer flasks with filtered, sterilised estuarine water f/2 medium under varying salinities[13] (10, 20, 30 & 40 psu). Standard protocol (25 °C, 1500 lux and 12:12 h light-dark cycle) was maintained throughout the

experiment[13]. Inoculum (10 % of the culture volume, 10×10^4 cells/mL) was prepared[14] and cell density was enumerated on alternate days[13].

Biochemical analysis

Cells were harvested in exponential and stationary phases by centrifugation (8000 rpm). Protein and carbohydrate contents were determined using the Lowry method[15] and Phenol-sulfuric acid method[16] using Bovine Serum Albumin (BSA) and glucose standard curves, respectively. Absorbance was measured with a UV-Visible Spectrophotometer at 660 nm (protein) and 490 nm (carbohydrate). Lipids were extracted (chloroform: methanol, 1:2) by the Bligh and Dyer method[17], then dried and weighed for Fatty Acid Methyl Ester (FAME) preparation[18]. FAME was analysed by a GC system equipped with a FAME WAX column TG-5MS. Statistical analysis used SPSS version 27, one-way ANOVA with Tukey's post hoc tests (P <0.05 for statistical significance).

Results and Discussion

The obtained sequences were deposited in the NCBI GenBank, and an accession number was acquired (OQ389490). The length of the edited and aligned Large Subunit (LSU) sequences of the 28S gene of *Halamphora* sp. was 784 bp. The BLASTn results in the GenBank database confirmed an identity of species with Bacillariophyta (MH810166) (97.20 %), a query cover of 100 % and an *E*-value of 0.

Growth kinetics revealed that maximum cell densities were observed at 30 psu, while higher salinities (40 psu) led to a decrease, as reported by Garcia[19]. *Halamphora* sp. showed exponential growth from day 5 – 15, reaching a maximum of 108×10^4 cells/ml on day 10. The stationary phase lasted from day 16 – 20, with a peak value of 98.33×10^4 cells/ml. At 10 psu, cell densities were comparable to those at 30 psu, measuring 95.33 and 90.66×10^4 cells/ml during log and stationary phase, respectively.

The effects of salinity on the diatom biochemical composition are tabulated in Table 1 and in Figures 1 & 2.

Table 1 — Fatty acid profiling (%) of *Halamphora* sp. in different salinities harvested during exponential and stationary phases of culture (*Nd = not detected)

Fatty acids (%)	Exponential phase				Stationary phase			
	10 psu	20 psu	30 psu	40 psu	10 psu	20 psu	30 psu	40 psu
C4:0 Butyric acid	61.33±1.87	77.26±1.33	55.63±1.19	61.66±3.05	66.43±0.40	14.13±0.15	14.86±1.30	42.06±1.75
C11:0 Undecanoic acid	Nd	Nd	1.86±0.05	Nd	Nd	Nd	Nd	Nd
C14:0 Myristic acid	Nd	Nd	3.96±0.25	3.76±1.13	Nd	3.23±0.20	4.76±0.45	3.23±0.20
C15: 0 Pentadecanoic acid	Nd	Nd	Nd	Nd	Nd	2.96±0.15	3.16±0.15	5.5±1.15
C16: 0 Palmitic acid	4.2±0.2	3.73±0.288	9.76±0.05	10.53±2.15	6.5±0.36	6.4±0.2	16.76±0.23	9.06±0.11
C17:0 Heptadecanoic acid	Nd	1.26±0.30	Nd	Nd	Nd	Nd	2±0.1	Nd
C18:0 Stearic acid	Nd	Nd	Nd	Nd	Nd	Nd	0.76±0.05	Nd
C 20:0 Arachidic acid	Nd	Nd	2.93±0.05	Nd	Nd	Nd	3.46±0.35	Nd
C23:0 Tricosanoic acid	5.4±0.26	Nd	2.06±0.05	Nd	2.06±0.05	8.33±0.28	14.23±0.41	11.36±0.35
C24:0 Tetracosanoic acid	Nd	Nd	Nd	Nd	Nd	Nd	0.53±0.20	Nd
SFA	70.93±2.13	82.26±1.48	76.23±1.09	75.96±0.77	75± 0.45	65.06 ±0.49	60.56± 1.00	71.23 ±0.75
C14:1 Myristoleic acid	4.73±1.20	Nd	Nd	Nd	8.36±3.09	11±0	Nd	Nd
C15:1 Pentadenoic acid	Nd	Nd	Nd	Nd	Nd	1.43±0.15	3.33±0.25	Nd
C16: 1 Palmitoleic acid	4.2±0.7	5.56±0.40	8.06±0.05	11.9±0.91	Nd	8.3±0	20.7±0.26	16.26±1.15
C17:1 Heptadenoic acid	5.43±1.06	Nd	Nd	Nd	6.96±1.70	Nd	Nd	Nd
C18:1 Oleic acid	4.4±1.25	Nd	4.2±0.60	Nd	Nd	Nd	3.4±0.81	Nd
C 22:0 Eucic acid	Nd	Nd	Nd	Nd	Nd	2.1±0.1	Nd	Nd
MUFA	18.76±1.04	5.56±0.40	12.26±0.60	11.9±0.91	15.33± 4.78	22.83 ±0.11	27.43 ± 0.75	16.26± 0.77
C18:2 Linoleic acid	Nd	Nd	3.16±0.15	Nd	Nd	Nd	3.76±0.65	Nd
C18:3 Gamma Linolenic acid	9.76±1.32	8.6±0.91	Nd	11.26±1.15	Nd	11.8±0	1.96±0.35	10.83±1.60
C20:3 Eicosatrienoicacid (EPA)	Nd	Nd	Nd	Nd	Nd	Nd	5.3± 0.26	Nd
C20:5 Eicosapentaenoic acid (EPA)	Nd	1.5±0.2	Nd	0.76±0.11	Nd	Nd	Nd	Nd
C22:6 Docosahexaenoic acid (DHA)	Nd	0.86±0.05	8.26±0.46	0.76±0.05	8.66±0.90	Nd	3.13±0.23	Nd
PUFA	9.76±1.32	10.96±1.00	11.43±0.58	12.8±1.32	8.66±0.90	11.8±0	14.16±0.30	10.83±1.60

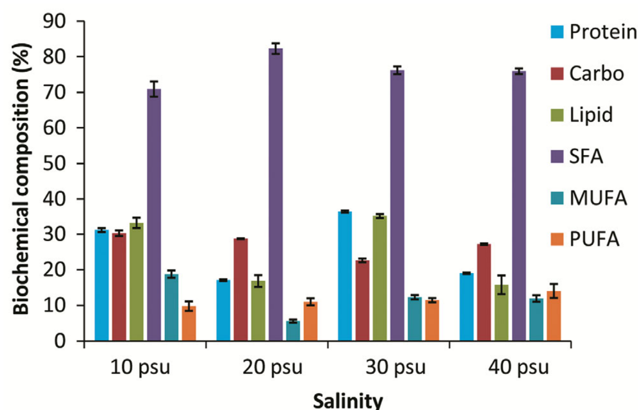

Fig. 1 — Biochemical composition (%) of *Halamphora* sp. cultured during exponential phase in different salinities

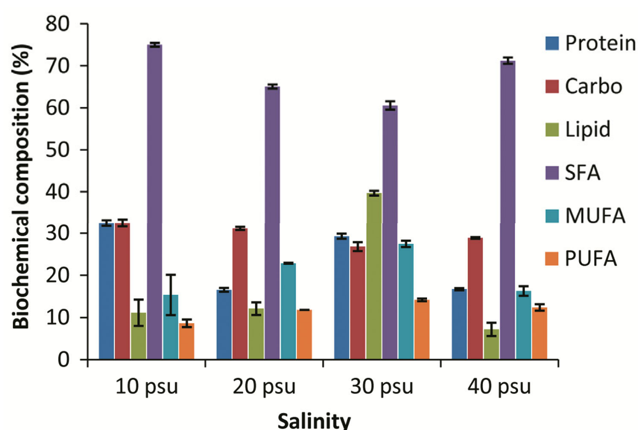

Fig. 2 — Biochemical composition (%) of *Halamphora* sp. cultured during stationary phase in different salinities

Protein production was influenced significantly ($P < 0.05$) during the logarithmic phase across all salinities. In the stationary phase, protein content differed considerably ($P < 0.05$) between 10 and 30 psu, but not between 20 and 40 psu. The highest protein content (36.78±0.35 %) was observed at 30 psu during the exponential phase, similar to previous reports for the diatom species *Thalassiosira weissflogii*[19,20].

Carbohydrate production elevated in the stationary phase (10 psu, 32.25±0.89 %). During exponential growth, levels varied significantly ($P < 0.05$) across salinities, though there was no significant difference between 30 & 40 psu salinities and 10 & 20 psu levels. Carbohydrate content was notably affected by salinity, aligning with results for another diatom, *Chaetoceros calcitrans*[21].

Lipid content of the isolate was similar across all salinity levels except 30 psu in the exponential and stationary phases. Lipid content at 30 psu salinity showed a significant difference ($P < 0.05$) compared to 20 and 40 psu. The highest lipid concentrations (39.67±0.58 %) were found at the stationary phase (30 psu). The lipid content decreased as the culture progressed to the stationary phase; this data is consistent with that of *T. weissflogii*[19].

Saturated Fatty Acids (SFA) were the dominant lipid class (60.56 – 82.26 % of total fatty acids), followed by Monounsaturated Fatty Acids (MUFAs) (5.56 to 27.43 %), and PUFAs (9.76 to 14.16 %), which was in compliance with the studies in *Monoraphidium braunii*[22]. Palmitic acid (C16:0) was the prominent SFA, while palmitoleic acid (C16:1) and oleic acid (C18:1) dominated MUFAs, with linolenic acid (C18:3) and DHA (C22:6) representing PUFAs. It was reported that biodiesel produced from *M. braunii* cultured under salt stress had more saturated and fewer unsaturated fatty acids, suitable for high-quality biodiesel, since SFAs encourage transesterifcation[22].

Saturated fatty acids content varied across all salinity levels in the stationary phase; however, in the log phase, it elevated to 82.26 % at 20 psu ($P < 0.05$). During the stationary phase, PUFA content showed detectable differences with other salinities, whereas MUFA remained similar at 20 and 30 psu.

The EN14214 biodiesel standard specifies that FAME should contain no more than 12 % linolenic acid and 1 % of fatty acids with four double bonds. Here the isolate conforms to these standards, as its linolenic acid content is below 12 %, which is in agreement with Thajuddin[23]. It was reported that the presence of PUFAs in algae can contribute to oxidation, and that adding antioxidants can mitigate this issue and maintain biodiesel quality[24].

Protein and lipid content were highest at 30 psu, while carbohydrate levels were at peak at 10 psu. Optimal growth and composition of this isolate occurred at 30 psu. Many microalgal species are tolerant to salinity regimes, and their chemical composition varies with salinity and culture age[25-28]. In this investigation, salinity and growth phase are the key influencing factors which could help to mould microalgae for biodiesel production and aquaculture nutrition.

Conclusion

This investigation carried out on the preliminary biochemical characterisation of *Halamphora* sp., collected and isolated from an estuary; however, identification of metabolites has been conducted

abroad recently[29]. Salinity fluctuations induce significant changes in the growth rate, biomass production and biochemical composition of *Halamphora* sp. Cell density, as well as carbohydrate, lipid, and protein contents were higher at low salinities, whereas high salinities negatively affected growth rate, cell density and organic composition. A high level of saturated fatty acids makes this species well-suited for biodiesel production, whereas suitable levels of PUFAs such as linolenic acid and DHA may enhance its applicability in both biodiesel and the aquaculture industry.

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Conflict of Interest

The authors declare that they have no conflicts of interest and all authors have given consent for publication.

Author Contributions

VSL: Conceptualization, formal analysis, funding acquisition, investigation, resources, software, roles/writing - original draft, and writing - review & editing; NA: Investigation, formal analysis, resources, roles/writing - original draft, and writing - review & editing; KSN, VA & SM: Investigation and Formal analysis; and MGS: Conceptualization, formal analysis, investigation, resources, software, supervision, roles/writing - original draft, and writing - review & editing.

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