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Nordic cyanobacterial and algal lipids: Triacylglycerol accumulation, chemotaxonomy and bioindustrial potential

Anita Santana-Sánchez | Fiona Lynch | Sema Sirin | Yagut Allahverdiyeva

Molecular Plant Biology, Department of Life Technologies, University of Turku, Turku, Finland

Correspondence
Fiona Lynch and Yagut Allahverdiyeva, Molecular Plant Biology, Department of Life Technologies, University of Turku, FI-20014 Turku, Finland.
Email: fiolyn@utu.fi and allahve@utu.fi

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Abstract

The ability to capture and convert sunlight, water and nutrients into useful compounds make photosynthetic microbes ideal candidates for the bio-industrial factories of the future. However, the suitability of isolates from temperate regions to grow under Nordic conditions is questionable. In this work, we explore the chemotaxonomy of Nordic strains of cyanobacteria and one green alga and evaluate their potential as raw materials for the production of lipid-based bio-industrial compounds. Thin-layer chromatography was used to identify the presence of triacylglycerol, which were detected in the majority of strains. Fatty acid methyl ester profiles were analysed to determine the suitability of strains for the production of biodiesel or the production of polyunsaturated fatty acids for the nutraceutical industry. The Nordic *Synechococcus* strains were unique in demonstrating fatty acid profiles comprised mostly C14:0, C16:0 and C16:1 and lacking polyunsaturated fatty acids. These properties translated to superior predicted biodiesel qualities, including cetane number, cold filter plugging point and oxidative stability compared to the other evaluated strains. Polyunsaturated fatty acids were detected at high levels (38–53%), with *Calothrix* sp. 336/3 being abundant in two essential fatty acids, linoleic and alpha-linolenic acid (21 and 17%, respectively). Gamma-linoleic acid was the predominant polyunsaturated fatty acid for the remaining strains (13–21%). In addition to assessing the potential of Nordic strains for bio-industrial production, this work also discusses issues such as taxonomy and predictive modelling, which can affect the identification of prospective high-performing strains.

1 | INTRODUCTION

Cyanobacteria and green algae are oxygenic photosynthetic microorganisms able to adapt to and flourish in a diversity of environments. They utilise many protective mechanisms, such as lipid unsaturation, to adjust to changing environments. Lipids, being major components of membranes, play a key role in protecting the photosynthetic machinery against environmental stresses such as strong light, salt, and extremes in temperature (Los et al., 2013). The Nordic region is generally characterised by low temperatures, with long, dark winters contrasting short, bright summers (Ferro et al., 2020). Using strains adapted to such unique conditions may be advantageous in the pursuit of robust algal production systems (Cheregi et al., 2019). Indeed, Long-chain polyunsaturated fatty acids (PUFAs) have been found to

Anita Santana-Sánchez and Fiona Lynch contributed equally to this study.

be higher in algae exposed to low temperatures or early growth stage in a recent study of cold-adapted marine microalgae (Schulze et al., 2019).

The importance of lipids in photosynthetic microbial evolution and environmental adaptation is reflected in several chemotaxonomic observations of fatty acid (FA) profiles (see, e.g., Sahu et al., 2013; Stamenković et al., 2020; Taipale et al., 2013) and can be observed in the clustering of related species according to geography (Temina et al., 2007). Indeed, the ability of cyanobacteria to change their features on prolonged exposure to a stable surrounding condition means that the stability of a particular strain itself can become uncertain (Komárek, 2018). It is not surprising then that one large scale study found chemotaxonomic discrimination to be restricted to phyla and class (Lang et al., 2011). Nevertheless, fatty acid profiling is certainly of value in the study of new and environmentally diverse strains which are continually being isolated and characterised for their potential employment in a growing bioeconomy. Indeed, understanding fatty acid profiles and plasticity of these are vital in establishing culture libraries for bioproduction potential.

Biodiesel, attractive as a 'drop in' fuel able to replace fossil fuel-based diesel can be obtained from the esterification of Eukaryotic algae, which are known to store excess carbon as triacylglycerol (TAG) in lipid droplets. In cyanobacteria, carbon storage is generally considered to be in the form of glycogen and/or polyhydroxyalkonoates (PHA), and the accumulation of neutral lipid TAG has long been reported to be absent in naturally occurring cyanobacteria (Hu et al., 2008). However, cyanobacterial TAGs have since been identified in the filamentous cyanobacterium *Nostoc punctiform* by thin-layer chromatography (TLC) analysis (Peramuna & Summers, 2014). More recently, TAG have also been isolated from the non-filamentous cyanobacterium *Synechocystis* sp. PCC 6803 (Aizouq et al., 2020). In their study, Aizouq et al. (2020) further identified the acyltransferase able to produce TAG in cyanobacteria as *slr2103*. A shift in paradigm regarding the possibilities of TAG accumulation in cyanobacteria presents new opportunities for lipid production in these organisms.

FAs are essential for human health and are thus valuable in nutritional and cosmetic industries. PUFAs have been promoted since the 1970s, when many Western countries recommended increased intake via consumption of vegetable- over animal-based fats (Ros, 2020). Supplements are one way to address diets that are suboptimal in regard to PUFA intake, such has been found in specific groups of European populations (Sioen et al., 2017). In the past 20 or 30 years, PUFAs have been produced using non-photosynthetic (or heterotrophically grown) algae and fungi as well as plant and fish oils. These products are commonly referred to as single cell oils (SCOs) and have been used, for example, in the production of infant formula (Ratledge, 2013). Examples of PUFAs commercially produced by SCOs include γ-linolenic acid (GLA, C18:3 omega-6), docosahexaenoic acid (DHA, C22:6 omega-3), arachidonic acid (ARA, C20:4 omega-6) and eicosapentaenoic acid (EPA, C20:5 omega-3). While industrial production has previously been dominated by heterotrophic cells, photosynthetic algae have also been determined to produce SCOs containing greater than 20% PUFAs, a level deemed to be commercially interesting (Ratledge, 2004).

In this work, we have performed a comprehensive lipid analysis of Nordic cyanobacteria and one green alga. We determine the presence of TAGs and evaluate the fatty acid profiles of the Nordic strains in order to determine their suitability to service in the blue bioeconomy for the future production of biofuels, which may be coupled with wastewater treatment; and/or bioproducts, for which a synthetic media is required.

2 | MATERIALS AND METHODS

2.1 | Strain collection and culture conditions

Eight native cyanobacteria and one green alga originally isolated from the Baltic Sea and Finnish lakes and held in the University of Helsinki Culture Collection (HAMBI) were used (Table 1). *Synechocystis* sp. PCC 6803 and *Chlorella vulgaris* (UTEX 265) (cyanobacterium and green alga, respectively) were also studied as model organisms. Cultures were maintained in standard BG-11 medium (Rippka et al., 1979) at pH 7.4 buffered by 5 mM HEPES, under continuous low light intensity (~15 μmol photons m^{-2} s^{-1} photosynthetic active radiation [PAR]) at 22°C without shaking.

Synthetic wastewater (synWW) medium was prepared based on BG-11 medium with adjusted concentrations of nitrate-N (NO_3 -N), ammonium-N (NH_4^+-N) and phosphate-P (PO_4^{+3}-P) as $NaNO_3$, NH_4Cl and K_2HPO_4 salt solutions to a final concentration of 3, 21 and 4 mg L^{-1} respectively. This resembles secondary effluent from municipal wastewater treatment plant, according to Shi et al. (2007). This composition was of interest for biodiesel analysis—a production process that may be coupled with wastewater treatment. However, synthetic wastewater was used in place of real wastewater as it is accepted that real wastewater may not be appropriate for some production platforms (e.g., nutraceuticals and cosmetics). Adjustment of pH to 7.4 and buffering with 5 mM HEPES were completed before autoclaving.

Pre-cultures were grown from a starting OD_{750} of 0.15 (Thermo Scientific, GENESYS 10S UV–Vis spectrophotometer) in 300 mL of synWW media contained in 500 mL Erlenmeyer flasks (Schott DURAN) for 7 days at 22°C with shaking (100 rpm) under atmospheric CO_2 and continuous light (50 μmol photons m^{-2} s^{-1}). Cells from the pre-culture were collected by centrifugation (9880 g, 22°C, 6 min) and used to prepare experimental cultures in 500 mL of fresh synWW media contained in 1 L Erlenmeyer flasks (Schott DURAN). Growth conditions were otherwise the same as pre-growth conditions, with all experiments cultured using 3 biological replicates. At the conclusion of experimental growth, total cells were harvested by vacuum filtration using stainless steel holders (Combisart®, Sartorius stedim) and nitrocellulose membranes (0.45 μm pore, Whatman® Protran®). Collected cells were immediately frozen in liquid-N_2 before undergoing lyophilisation with a freeze dryer (Millrock Technology). Lyophilised biomass was divided into separate portions as required

TABLE 1 List of native algae and cyanobacteria species from the University of Helsinki Culture Collection (HAMBI) with former (used in Lynch et al., 2015) and current catalogue IDs

	HAMBI culture collection UHCC identifier		
	Former catalogue #	Current catalogue #	Taxonomy
Green alga	UHCC0027	UHCC0027	*Scenedesmus* sp.
Cyanobacteria	1TU44S8	UHCC0492	Unknown
	1TU21S5	UHCC0543	*Synechococcus* sp.
	0TU24S4	UHCC0527	
	1TU39S1	UHCC0524	
	SYKE2088A	UHCC0582	*Microcystis* sp.
	SYKE695	UHCC0419	
	0TU37S4	UHCC0374	*Snowella litoralis*
			Calothrix sp. 336/3

for in situ transesterification (10 mg freeze-dried weight, single replicate) and total lipid determination (10 mg freeze-dried weight, biological triplicate). Extracted lipids obtained from total lipid determinations were then run on TLC plates (biological triplicate, with a representative plate being presented).

2.2 | Total lipid content

Total lipid content was determined according to Ryckebosh et al. (2012), using a solvent system consisting of chloroform:methanol:water. Briefly, approximately 10 mg of lyophilised biomass was vortexed in a glass tube with 400 μL methanol for 30 s. After chloroform (200 μL) and MQ-water (40 μL) addition, the sample was vortexed again. The sample was vortexed (30 s) again with 200 μL chloroform and 200 μL MQ-water and then centrifuged at 2000 rpm for 10 min. The upper layer was removed, and the lipid layer dried by passing through a column of anhydrous sodium sulphate. The pellet was then re-extracted with chloroform: methanol 1:1 (400 μL) and MQ-water (120 μL) using the same procedure. Solvents were removed at 40°C under a slow stream of nitrogen and gravimetric yields from both extractions were summed to determine the total lipid content. Analysis was performed in duplicate.

2.3 | Lipid composition

Lipid composition was determined by thin-layer chromatography (TLC) separation on silica gel plates (20′ 20 cm in size, Sigma-Aldrich, Cat#2737B25). The solvent system consisted of hexane: diethyl ether: acetic acid (80:20:1 v/v/v with polarity indices' of 0.1/2.8/6.2, respectively) to allow the separation of both polar and non-polar lipids. The TLC developing chamber contained 350 mL of the solvent system and kept closed for 1 h before the sample run to allow liquid–vapour phases to reach equilibrium. Dried lipids, obtained after total lipid content determination, were dissolved in 250 μL of hexane and stored at −20°C before application on TLC plates. The total lipid content applied was adjusted to 30 μg for all strains and the run was terminated at 1 h. Lipid identification was based on the migration profiles of a standard mixture of DAG: Glycerol 1,2(3)-dihexadecanoate (Sigma-Aldrich, Cat#D2636), FFA: Hexadecanoic acid (C16:0, Sigma-Aldrich, Cat.#76119) and a TAG mixture: Tricaprylin, Tricaprin, Trilaurin, Trimyristin and Tripalmitin (C8:0, C10:0, C12:0, C14:0 and C16:0, respectively, Sigma-Aldrich, Cat# 17811-1AMP).

Spots were visualised by the use of iodine vapour or by spraying 0.5% primuline solution (in 80% acetone) and placing the dried TLC plate under UV light.

2.4 | *In silico* analysis of slr2103

BLAST searches of the KEGG database (https://www.kegg.jp/kegg/kegg2.html) were used to find orthologs of *slr2103* in the published genome of the *Calothrix* sp. 336/3 native strain (Isojärvi et al., 2015). The remaining native strains, for which genome sequences were unavailable, were analysed based on the highest scoring ortholog in the genus of interest (*Microcystis* or *Synechococcus*). Amino acid sequences translated from the orthologs identified were then aligned with that of translated *slr2103*.

2.5 | Fatty acid methyl ester composition

Fatty acid methyl ester (FAME) composition was determined using whole biomass in situ transesterification as detailed in Van Wychen et al. (2016). The single-step extraction and derivatisation of 10 mg of dried biomass was performed in 1.5 mL amber-glass vials by addition of 200 μL of a Chloroform: Methanol (2:1, v/v) solvent system, followed by 300 μL of the acid catalyst solution 0.6 M HCl:Methanol. Reaction occurred at 85°C (1 h, no shaking) and separation of the lipid phase was achieved by adding 1 mL hexane. FAME was separated on an Agilent 7890C GC equipped with an Agilent Innowax (30 m × 0.32 mm × 0.5 μm, 19,091 N-213, Agilent) column and detected by 5975C inert mass spectrometer (MS) (Agilent, USA). One microlitre of sample was injected at 250°C using 6000 μL min^{-1} constant flow. The carrier gas was Helium at 1.4799 psi. The column

temperature programme started at 50°C, held for 3 min, then increased to 250°C at 15°C min^{-1}, where it was finally maintained for 10 min. The MS was run in Mode Scan (low: 35.0 m/z; high: 550.0 m/z). EMV mode gain factor was15.0 and MS source temperature was 230°C, with MS quad at 15°C. For the accurate identification and quantification of the FAME composition in samples, a 37- component standard FAME mix (C4:0–C24:0) (Sigma Aldrich #18919- 1AMP) was used, with weight % of individual FAME specified in the product data sheet. Methyl tridecanoate (C13:0ME, Sigma Aldrich #91558- 5ML) was employed as an internal standard (Van Wychen et al., 2016).

2.6 | Analysis of FAME composition

Reconstructed ion chromatograms (RIC) were obtained based on the identification of FAMEs using specific ions (m/z). Features including chain length, unsaturation degree, and location of double bonds were considered in the selection of representative m/z ions. Three specific m/z ions were selected to fully identify each FAME component in the 37-standard FAME mix and filter the total ion chromatogram (TIC). Final validation was obtained by comparing the mass spectra with the NIST MS Search 2.0 library.

Relative abundance for each FAME was calculated as follows:

$$\%\text{Relative Abundance} = (\text{Area of peak FAME} \,/\, \text{Total area for all FAMEs}) \times 100\%.$$

2.7 | Prediction of biodiesel properties

The prediction of the quality parameters for a prospective biodiesel from native photosynthetic microbes was based on their FAME composition. Biodiesel properties were estimated in silico using BiodieselAnalyzer© Ver. 1.2 as described in Talebi et al. (2014) (available at "http://www.brteam.ir/biodieselanalyzer").

2.8 | Statistical analysis

FAME data (% relative abundance) was used in non-parametric multidimensional scaling (NMDS) analysis (Euclidean distances) and SIMPER analysis using Past 4.04 free statistical software (Hammer et al., 2001).

3 | RESULTS

3.1 | Nordic cyanobacteria can produce TAG, likely via *slr2103* orthologs

TLC analysis undertaken on a selection of Nordic cyanobacterial strains, including unicellular and filamentous cell types, confirmed the presence of TAG in the majority of the strains (Figure 1). However, two of the three *Synechococcus* strains (0543 and 0527) did not exhibit clear TAG spots. The most diverse range of TAG was observed for strain UHCC0492, the cyanobacteria containing the highest neutral lipid content determined via flow cytometry (Lynch et al., 2015), while the native filamentous *Calothrix* sp. 336/3 appeared to have the highest content of TAG among cyanobacterial strains. The TAG profiles of cyanobacteria and algae differed, with algae demonstrating greater TAG content and cyanobacteria demonstrating longer carbon chains. The algae were sampled at a more advanced growth phase than cyanobacteria (refer Figure S1) but were unlikely to have reached stationary growth or nutrient deprivation (Lynch et al., 2015). There were no clear trends observed between the growth stage of cyanobacteria (Figure S1) and TLC profile. A larger amount of TAG may have contributed to the greater lipid content observed for algae over cyanobacteria (Figure S2). However, the absence of TAG in *Synechococcus* 0543 and 0527 did not result in noticeably lower lipid content as compared to the other cyanobacteria (Figure S2). Indeed, the diversity of lipids contributing to the total lipid content can be observed in the TLC plate, which was run using the total lipid extract (Figure 1).

The *Synechocystis* sp. PCC 6803 control strain used in our experiments was also used by Aizouq et al. (2020) to identify the diacylglycerol acyltransferase (DAGAT) responsible for TAG formation. We identified an ortholog of *slr2103* in native *Calothrix* sp. 336/3 (IJ00_05940; 61% sequence identity) for which a genome is available (Isojärvi et al., 2015). Since genomes are not available for the remaining native strains, IJ00_05940 and orthologs of the highest sequence identity from the genus of *Microcystis* (B5D77_04030; 62%) and *Synechococcus* (SYNPCC7002_A0918; 63%) were aligned with *slr2103*, highlighting strong conservation between the sequences (Figure 2). The slr2103 amino acid sequence was found to contain the Pfam domain PF03982.13 (DAGAT), which was also conserved in the orthologous amino acid sequences. Targeted searches against the published genomes of *Synechococcus* sp. strains PCC 7942 and PCC 6301 failed to detect *slr2103* orthologs. These strains were chosen as representatives of Group 1 *Synechococcus* strains (to which UHCC0543 and UHCC0527 also belong), according to the Kenyon-Murata classification system (Los & Mironov, 2015).

3.2 | FAME profiles of Nordic cyanobacteria provide useful chemotaxonomic information and indicate bioproduction potential

The FAME profiles obtained from the native cyanobacteria and algae exhibited chemotaxonomic trends, whereby the *Synechococcus* genus was clearly differentiated based on NMDS and cluster analysis (Table 2, Figure 3). The total lipid content of the strains ranged between 6.7 and 23.5% of the dry weight. As in our previous work, the green algae generally showed higher lipid content than the cyanobacteria (Figure S2 and Lynch et al., 2015). All strains showed the presence of saturated fatty acids (SFAs) and monounsaturated fatty

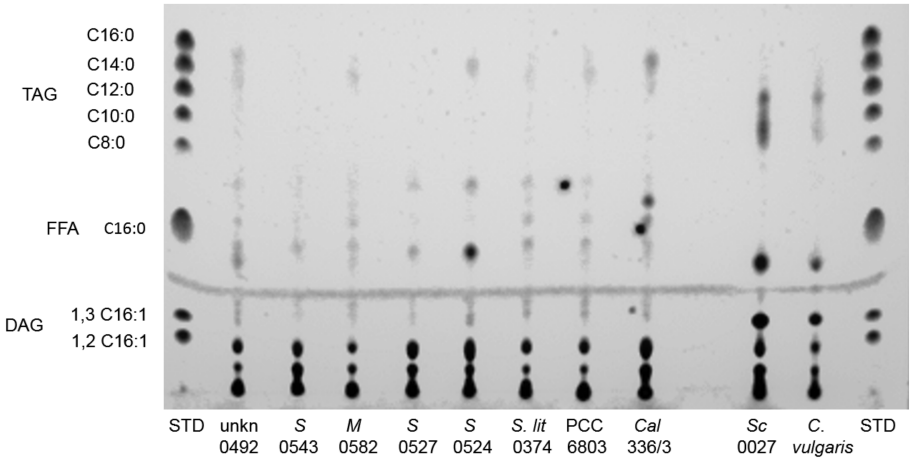

FIGURE 1 Representative TLC separation of lipids extracted from eight strains of cyanobacteria (left) and two strains of algae (right) visualised using iodine vapour. Loading was 30 μg of extracted lipid for each sample. *C*, *Chlorella*; *Cal*, *Calothrix*; *M*, *Microcystis*; PCC, *Synechocystis* sp. PCC 6803; *S*, *Synechococcus*; *S. lit*, *S. litoralis*; *Sc*, *Scenedesmus*; STD, standard FA mix; unkn, unknown taxonomy

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slr2103          ------MLRATSDRSPLFECNP-----ALDGWSLGGRDPETIKRLMPFWEWFYKYYFRVQTSGWENLPPGQ-VLLVGSHN
SYNPCC7002_A0918 ----------MPLFPPFPPLDP-----PLDGWSLEGRDPETIRRLLPLWEWLYKFYFRVETSGWENLPTDEPVLGVGSHN
IJ00_05940       ----------MEKHAEQPSHSQ-----KKPGWSLDARDVEFIKSLMPIWGFLYDYYFRVKTSGWENVPTNEKVLFVGSHN
B5D77_04030      MQIFSSFVSSTATFAELSLQAAKAPVDTLTGWSLEDRDPQVIEKFVPLLDWFYHHYFRVKTDGWENIPPSGQVLFIGSHN

slr2103          GGLSSPDMVMMIYDWFRCQGLHRPVYGLMHPTVWKMSRPLAELAVKVGAVQAHPKMAIAALQSGASVLVYPGGAQDVFRP
SYNPCC7002_A0918 GGLAAPDMWMMMYDWFRRYGVERPTYGLMHRNIWLAFPELAKIAVKTGAVQAHPKMAIAALKSGANVLVYPGGGQDVFRP
IJ00_05940       GGLASPDMQMMMYDWFRRFGVERPVYGLMHPKVAEVSPEVAELAAKVGAITAHPKMAIAAFRSGASVLVYPGGIQDVFRL
B5D77_04030      GGLAAPDMFMMMYDWFQRFGSDRLVYGLMDSRVWRVFPSQAHLVAQMGAIHAHPKMAIAALNSGASVLIYPGGATDVFRP

slr2103          FSQRHEIYFAGRKGFIKLALRQGVPIVPAIAVGSHETLLVMGDCYDQVK-YLHDLGMPWLLDVDPVVFPIYLGLPWGLAL
SYNPCC7002_A0918 HAQRNQIYFAERRGFIKLALRQGVPIVPLISWGAHDSIFVIEDIYEPLKGFLKAFNLPWLFNIDPEVFPIYLGLPWGVAF
IJ00_05940       HSLRDKIYFHNRHAFIKLALREQVPIVPAISWGAHDTLIVLADLHKIIQ-KIHSLGIPWLFNVDPEVFPVYLGLPWGIGI
B5D77_04030      HSLRNKIHFAGNQAFVKLALQYEVPIIPAISHGAHSTLFVLDDIYPQLK-ELHKKGMPWPFGIDPGTCPIYFGLPWGLAI

slr2103          GPLPHVPLPLTMHTRICPPIYFERYGNDAAKDKDYVEQCYQLVKTEMQRELDQLAETANILPQLNFL
SYNPCC7002_A0918 GPLPNFPLPTKMYTRVCPPIRFEKYGRAAAGDRPYVEACYQRVLGEMQWELDQLIAEVESRPKSCP-
IJ00_05940       GPLPNIPFPVQIYTRICPPIIFERYGREAAGDAAYVDACYQIVVSKMQQELDELIEGSRE-------
B5D77_04030      GPLPNIPLPVPIHTRVCPPIIFERYGKKAARDRRYVRECYEKVCYLMQQQLDQLVAANSP-------
```

FIGURE 2 Alignment of translated amino acid sequences orthologous to translated *slr2103* for *Synechococcus* PCC7002 (SYNPCC7002_A0918), *Calothrix* sp. 336/3 (IJ00_05940) and *Microcystis* sp. MC19 (B5D77_04030)

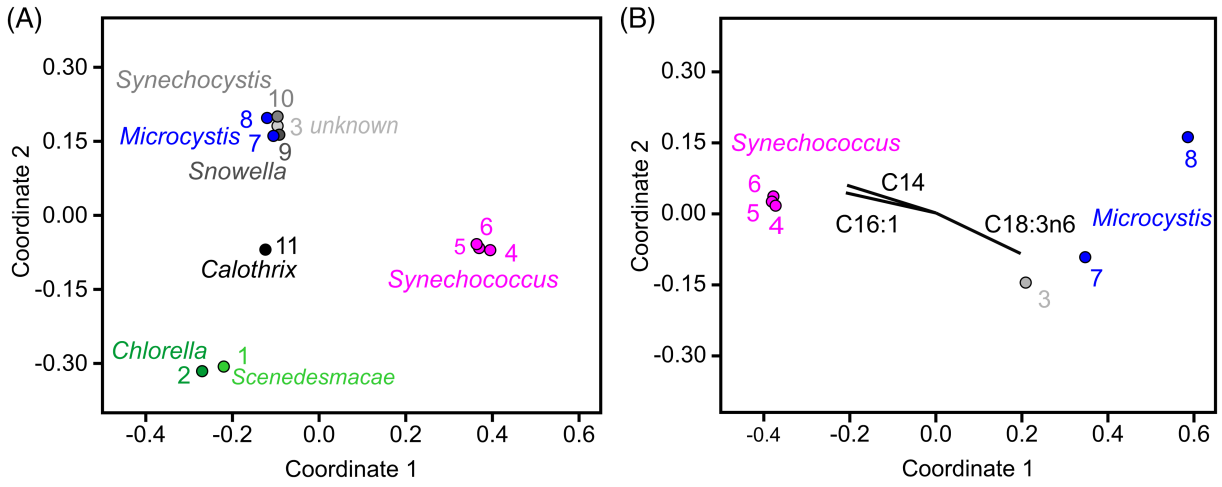

FIGURE 3 (A) Two dimensional representation of non-metric multidimensional scaling (NMDS). Stress value = 0.1. Green algae (indicated in green) are *Scenedesmacae* UHCC0027 (1) and *Chlorella vulgaris* (2). Cyanobacterium UHCC0492 (3) is of unknown taxonomy. Cyanobacteria belonging to the *Synechococcus* genus (magenta) are: UHCC0543 (4); UHCC0527 (5); UHCC0524 (6). Cyanobacteria belonging to the *Microcystis* genus (blue) are: UHCC0582 (7); and UHCC0419 (8). Remaining cyanobacteria are: UHCC0374 *Snowella litoralis* (9); *Synechocystis* sp. PCC 6803 (10); and *Calothrix* sp. 336/3 (11). (B) NMDS of *Synechococcus* and *Microcystis* grouped strains (UHCC0492 included in *Microcystis* group) with overlayed vectors indicating the direction of FAs (environmental variables) determined to have the greatest contribution to the difference between the two groups as determined by SIMPER analysis. Vectors are plotted from the origin, with lengths arbitrarily scaled. Stress value = 0.0

TABLE 2 Fatty acid methyl ester (FAME) profiles. Relative abundance of FAMEs (% of total), FAMEs not detected are indicated by a dash (–)

	Green algae		Cyanobacteria								
Taxonomy	*Scenedesmus* sp.	*Chlorella*	Unknown	*Synechococcus* sp.			*Microcystis* sp.		*Snowella litoralis*	*Synechocystis*sp.	*Calothrix* sp.
Fatty acid (%)	UHCC0027	*vulgaris*	UHCC0492	UHCC0543	UHCC0527	UHCC0524	UHCC0582	UHCC0419	UHCC0374	PCC6803	336/3
Saturated	26.88	33.46	45.07	51.04	53.90	51.84	39.45	50.62	30.84	45.25	35.60
C14:0	–	–	–	22.10	22.66	21.05	–	–	–	–	0.19
C15:0	–	0.20	0.16	0.26	1.10	0.44	1.36	0.29	–	–	0.50
C16:0	23.30	28.86	44.19	28.20	27.55	29.91	37.07	48.14	30.84	45.13	34.74
C17:0	0.78	2.99	0.41	0.49	1.37	0.44	1.02	0.21	–	0.12	–
C18:0	0.35	0.46	0.31	–	1.22	–	–	1.98	–	–	0.17
C20:0	0.34	–	–	–	–	–	–	–	–	–	–
C22:0	1.35	–	–	–	–	–	–	–	–	–	–
C23:0	–	0.43	–	–	–	–	–	–	–	–	–
C24:0	0.75	0.52	–	–	–	–	–	–	–	–	–
Monounsaturated	20.49	15.95	15.15	48.96	46.10	46.11	16.75	9.35	17.21	9.95	26.48
C14:1	–	–	–	3.97	2.26	3.35	–	–	–	–	–
C15:1	–	–	–	–	0.76	0.79	–	–	–	–	–
C16:1	4.47	1.78	5.90	36.58	32.90	35.79	7.20	2.84	9.06	7.10	17.35
C17:1	0.53	0.70	1.12	1.14	0.93	1.22	2.55	0.75	4.08	2.84	0.64
C18:1 n9	14.41	13.47	8.14	6.07	8.51	4.19	5.10	3.83	–	–	7.54
C20:1	1.09	–	–	–	–	–	–	–	–	–	–
C22:1 n9	–	–	–	1.19	0.74	0.78	1.89	1.92	4.07	–	0.94
Polyunsaturated	52.63	50.59	39.78	0	0	2.05	43.80	40.03	51.95	44.81	37.92
C16:4	9.56	0.62	0.79	–	–	–	–	–	–	–	–
C18:2 n6 (LA)	10.08	18.44	9.46	–	–	2.05	12.24	5.15	10.61	9.02	21.06
C18:3 n3 (ALA)	24.59	30.29	7.82	–	–	–	9.04	9.37	10.30	9.09	16.85
C18:3 n6 (GLA)	2.67	0.73	15.97	–	–	–	15.98	12.89	21.13	18.38	–
C18:4	5.73	0.51	3.42	–	–	–	4.95	9.86	6.10	5.22	–
C20:3 n3	–	–	1.11	–	–	–	–	1.36	–	–	–
C20:3 n6	–	–	1.20	–	–	–	1.59	1.41	3.81	3.10	–

TABLE 3 Biodiesel properties of the studied strains estimated using BiodieselAnalyzer© Ver. 1.2 (described in Talebi et al., 2014)

		Green algae		Cyanobacteria								
	Taxonomy Biodiesel properties	*Scenedesmus* sp. UHCC0027	Chlorella vulgaris	Unknown UHCC0492	UHCC0543	*Synechococcus* sp. UHCC0527	UHCC0524	*Microcystis* sp. UHCC0582	UHCC0419	Snowella litoralis UHCC0374	*Synechocystis* sp. PCC 6803	*Calothrix* sp. 336/3
Combustion quality and oxidative stability	SV	184.11	202.26	201.13	209.95	212.8	210.05	196.96	199.01	184.05	198.27	206.91
	IV (<120)	132.24	134.09	108.03*	41.98*	40.50*	43.21*	120.62	112.77*	136.7	117.79*	108.36*
	CN (>51)	46.19	43.11	49.13	62.85*	62.84*	62.56*	46.87	48.35	45.20	47.33	48.30
	DU	106.11	115.21	87.38	42.65	41.41	44.08	96.72	81.21	105.34	90.52	100.71
	APE	100.55	113.43	81.48	6.07	8.51	8.29	89.52	78.37	96.28	83.42	83.36
	BAPE	65.28	80.5	57.04	0	0	2.05	62.28	49.67	73.47	63.96	54.76
	OS (>8 h)	5.75	4.97	6.14	0	0	0	5.76	6.89	5.40	5.82	5.7
Cold flow properties	LCSF	6.37	4.16	4.57	2.82	3.37	2.99	3.71	5.8	3.08	4.51	3.56
	CFPP (<−5 °C)$^{+}$	3.54	−3.41	−2.12	−7.62*	−5.89*	−7.08*	−4.82	1.74	−6.8*	−2.31	−5.29*
	CP	7.26	10.19	18.25	9.84	9.5	10.74	14.51	20.33	11.23	18.75	13.28
Physical properties	HHV	35.33	38.6	37.55	36.46	37.12	36.54	36.81	37.04	34.46	36.82	38.58
	ν (3.5–5)	1.1	1.2	1.17	1.13	1.16	1.13	1.12	1.14	1.01	1.12	1.21
	ρ (0.86–0.9)	0.79	0.86*	0.84	0.81	0.83	0.81	0.82	0.83	0.77	0.82	0.86*

Note: Where applicable, EN 14214 standards are stated in parenthesis. Values meeting the relevant EN 14214 standard are indicated by an asterix (*) ($^{+}$for CFPP, a value of −5°C was used on the basis of the lowest summer time value).
Abbreviations: APE, allylic position equivalents; BAPE, bis-allylic position equivalents; CFPP, cold filter plugging point (°C); CN, cetane number; CP, cloud point (°C); DU, degree of unsaturation; HHV, higher heating value; IV, iodine value (g I_2); LCSF, long-chain saturated factor; OS, oxidative stability (h); SV, saponification value; ν, kinematic viscosity (mm^2 s^{-1}) at 40°C; ρ, density (g cm^{-3}) at 20°C.

acids (MUFAs), which ranged from 27–54% and 9–49% of total fatty acids, respectively (Table 2). PUFAs were present at moderate compositions (38–53%) for all strains, except for the *Synechococcus* species, which had either none, or negligible, PUFA content. The most abundant *Synechococcus* FA was C16:1, which, at 33–37% composition, was far greater than those found in all other strains and resulted in distinction of the genus on the basis of MUFA levels alone. Indeed, C16:1 was found to make the greatest contribution to the dissimilarity observed when the *Synechococcus* group was compared to *Microcystis* using SIMPER analysis (Figure 3B). The abundance of the medium chain SFA C14:0 was also characteristically high (~22% Table 2) and contributed to the distinction of this genus (Figure 3B).

As for *Synechococcus* sp., the FA distribution patterns of *Microcystis* sp. demonstrated intra-genus similarities, although with limited sample size. *Microcystis* FA profiles clustered with the unidentified cyanobacterium UHCC0492 and with *Synechocystis* sp. 6803 (Figure 3B). The FAME profiles were mostly dominated by the SFA C16:0 (37–48%), followed by the PUFA C18:3 (~25%). The C18:3 PUFA was the second greatest contributor to dissimilarity between *Microcystis* and *Synechococcus* strains as analysed using SIMPER (Figure 3B). In performing SIMPER analysis, UHCC0492 was grouped with the *Microcystis* strains on the basis of its similarity and to equalise the sample size of the groups. At 9–17%, the MUFA compositions of *Microcystis* sp. were overall much lower than that of *Synechococcus* and were characterised by low to moderate concentrations of C16:1, C17:1, C18:1 and C22:1. While MUFAs were less diverse than in *Synechococcus* sp., they were more diverse than in the other cyanobacteria studied.

The FA distribution pattern of the studied cyanobacterial strains UHCC0492 (taxonomy unknown), UHCC0374 (*Snowella litoralis*) and *Synechocystis* sp. PCC 6803 showed similar trends to *Microcystis* strains (Figure 3A,B). The main fatty acid was C16:0 (31–45% of FAMEs), with C18:3 and C18:2 also prominent. In contrast to the rest of cyanobacterial species, UHCC0374 (*S. litoralis*) and *Synechocystis* sp. PCC 6803 did not show any C18:1 content. The native *Calothrix* strain, the only filamentous strain studied, showed a similar dominance of C16:0 (35%), but higher levels of C18:2 (21%) than the other cyanobacteria and thus could be clearly distinguished from the other strains (Figure 3A). The green algae UHCC0027 and *C. vulgaris* clustered together (Figure 3A), with higher PUFA content than all cyanobacteria, except *S. litoralis* (Table 2). They also contained a greater diversity of SFAs, which included longer carbon chains up to C24, although these higher C SFAs were only detected at low levels compared to C16:0 (Table 2).

Employment of the BiodieselAnalyzer© tool (Talebi et al., 2014) demonstrated the different biodiesel potentials of the Nordic strains, with *Synechococcus* strains demonstrating the best overall potential by meeting iodine value (IV) and cetane number (CN) requirements of the EN 14214 (Table 3) and having the lowest cold filter plug point (CFPP) values. As is often the case with biodiesel, the oxidation stability (OS) was lower than required, although for *Synechococcus* strains this was surprising, based on a lack of unsaturation (Table 2) and BAPE values (Table 3) which actually indicated high resistance to oxidation. The physical properties of viscosity (ν) and density (ρ) did not meet European standard requirements, although the viscosity values were closer to the American standard (ASTM D6751) values of 1.9–6.0 $mm^2\ s^{-1}$. Viscosity increases with increasing chain length and decreases with increasing degree of unsaturation. Viscosity becomes more important at low temperatures where it increases exponentially, decreasing flow and affecting engine performance (Knothe, 2009). Both viscosity and density standards have been met previously by the green alga UHCC0027 under different growth conditions (Jämsä et al., 2017), thus it is possible to alter the FAME profile in this way.

4 | DISCUSSION

4.1 | A paradigm shift for the production of TAG in cyanobacteria

Cyanobacteria have long been thought to lack the natural ability to accumulate TAG (see, e.g., Hu et al., 2008; Sheehan et al., 1998). While all fatty acids are available for conversion to biodiesel via direct, whole biomass transesterification (Wahlen et al., 2011) the yield of lipids can be largely increased by the accumulation of storage lipids in the form of TAG. Cyanobacterial lipid droplets, potentially containing TAG, have been observed in microscopy images (Edwards et al., 1968; van de Meene et al., 2006; Wolk, 1973). However, it was less than a decade ago that TAGs were first confirmed in lipid droplets of filamentous cyanobacterium *Nostoc punctiform* (Peramuna & Summers, 2014). Furthermore, it has only been recently that TAG presence has been reported in unicellular cyanobacteria (Aizouq et al., 2020). Importantly, Aizouq et al. (2020) also identified the *slr2103* gene coding for the acyltransferase responsible for the conversion of diacylyglycerol (DAG) to TAG, thus revealing new bioengineering possibilities. We have previously observed the presence of neutral lipids in unicellular strains of native cyanobacteria selected for analysis from the UHCC (Lynch et al., 2015). While flow cytometry and total lipid techniques showed lower levels of neutral lipids than the algae evaluated in the same work, the relative lack of regard for cyanobacterial TAG in the literature and a lack of proposed biosynthetic pathway prior to the work of Aizouq et al. (2020), warranted the confirmation of TAG presence in the Nordic cyanobacterial strains presented in this study. The confirmation of TAG presence in some of these strains supports the nascent paradigm shift towards cyanobacteria as potential TAG producers. Furthermore, our findings (Figure 2) suggest the role of *slr2103* orthologs as diacylglycerolacyltransferases (Pfam domain PF03982.13 DAGATs) functioning in the production of cyanobacterial TAG, as proposed by Aizouq et al. (2020). Indeed, the *slr2103* ortholog identified for *Calothrix* sp. 336/3 in this work is a potential candidate for genetic approaches to improving TAG accumulation in this Nordic strain. This could be combined with lipid accumulating conditions, such as those demonstrated for *N. punctiforme* including exogenous fructose addition, altered nitrogen source and advanced growth phase (Peramuna & Summers, 2014).

4.2 | FAME plasticity based on taxonomy, geographical origin and culture conditions

Understanding chemotaxonomy and its relationship to lipid properties of photosynthetic microbes are important in establishing screening programmes aimed at bioprospecting and/or evaluating existing culture collections, such as the HAMBI culture collection sampled here. The Nordic strains of cyanobacteria evaluated are geographically related, being isolated from Finnish freshwater lakes and, in the case of green alga UHCC0027, a brackish coastal area.

The strongest intra-genus similarities were demonstrated by the *Synechococcus* sp. This genus was clearly differentiated due to the almost complete absence of PUFAs and dominance of MUFAs (Table 2). As freshwater strains, *Synechococcus* sp. UHCC0543 and UHCC0527 are classified as Group 1 cyanobacteria according to the Kenyon-Murata system (Kenyon, 1972; Kenyon et al., 1972; Los & Mironov, 2015; Murata et al., 1992). Strain UHCC0524 was differentiated from the other *Synechococcus* strains in both the presence of TAG (Figure 1) and the detection of a small amount of PUFA (C18:2 at 2% relative abundance), which makes Kenyon-Murata classification unclear. The PUFA content may have been due to the presence of contaminating organisms (of the native strains only *S. litoralis* and UHCC0027 are axenic), but was not sufficient to distinguish it from the other *Synechococccus* strains on the basis of chemotaxonomy, as analysed by NMDS (Figure 3A). The *Synechococcus* genus is known to be polyphyletic and lack clear taxonomic structure, with a systematic revision most recently proposed by Salazar et al. (2020). Given the variability in lipid profiles of the *Synechococcus* genus, it is interesting that Group 3α *Synechococcus* sp. PCC 7002 was found to have an *slr2103* ortholog (SYNPCC7002_A0918, Figure 2), but we did not find orthologs in published genomes of *Synechococcus* sp. strains PCC 7942 and PCC 6301. The latter strains are listed in Los and Mironov (2015) as representative of Group 1 cyanobacteria. This suggests that a lack of TAG for Group 1 Nordic strains UHCC0543 and UHCC0527 (Figure 1) may be related to a lack of functional DAGAT encoded by an *slr2103* ortholog, although genomic sequencing of the Nordic *Synechococcus* strains would be required to further explore this, and to determine the presence of an *slr2103* ortholog in the TAG-containing *Synechococcus* UHCC0524 strain.

Native *Calothrix* sp. 336/3, the only filamentous strain evaluated in this work, was clearly distinguished from the other cyanobacterial strains studied (Figure 3A). However, the organisation or complexity of cyanobacterial cells is not known to correlate with FA composition generally (Los & Mironov, 2015). The *Calothrix* FAME profile showed strong similarity to the profile of a native strain isolated from Northern India, which also had a FAME profile dominated by C16:0 (at 46%, 11% more than in this study), followed by C18:2 and C16:1 (both 18%; approximating the results of this study, Deshmukh et al., 2019). The main difference between the FAME profiles of the two geographically distant strains was distribution of unsaturation. The Finnish isolate evaluated in this study demonstrated a relatively high degree of unsaturation, notably in the form of C18:3 (17%, Table 2), which was not detected in the Indian isolate. The Indian isolate in turn demonstrated much higher SFA content (∼55%, Deshmukh et al., 2019). This higher PUFA content in our strain is possibly due to cold climate adaptation, with increased unsaturation required to maintain membrane fluidity under cold temperatures (Hazel, 1995; Los & Murata, 2004). However, the presence of C18:3 was also widespread among the eight *Calothrix* strains from the culture collection of Göttingen University, Germany (SAG) despite isolation from a variety of climatic conditions around the world, (Lang et al., 2011; https://sagdb.uni-goettingen.de/). This may be representative of maintenance in a culture collection, which presents opportunity for strains to adapt to lab temperatures and growth conditions. The *Calothrix* evaluated by Deshmukh et al. (2019) was not only isolated from a warmer climate, but also grown at a temperature approximately 8°C higher than in this study. Thus, experimental growth conditions should also be considered in FAME profiling and may be useful in tuning FAME profiles to the targeted production of particular compounds.

4.3 | Group 1 Nordic *Synechococcus* isolates have distinctive biodiesel properties

Biofuel properties are dependent on the FAME profiles of strains, which can vary greatly. While not all requirements of EN 14214 could be met by the Nordic strains, this is a common finding for predictions of unblended algal biodiesel. Indeed, it has been said that almost all FAME based biofuels face performance issues (Knothe, 2009).

Medium-chain saturated and monounsaturated FAs of C12–C18 have been described as ideal for the production of biodiesel from cyanobacteria, this is on the basis of CN and IV which are indicators of ignition and combustion quality (Sarsekeyeva et al., 2015). The Nordic *Synechococcus* strains evaluated here comprised predominantly C14 and C16 saturated and monounsaturated FAs (Table 2), which indeed resulted in high enough CN and low enough IV (Table 3) values required to meet standards. The *Synechococcus* strains had exceptionally high CNs with values over 60, while the other Nordic strains were in the range (40s) predicted for most algal oils (Knothe, 2012).

Most of the predicted properties generated using the BiodieselAnalyzer© tool (Talebi et al., 2014) matched expectations based on the FAME profiles. For example, high SFA content and a lack of PUFAs (i.e., a lower degree of unsaturation comparative to other strains) resulted in lower IV values. These properties generally indicate superior oxidative stability (Rocha Jr et al., 2019). However, allylic position equivalents (APE) and bis-allylic position equivalents (BAPE) are better indicators of oxidative stability than IV (Knothe, 2002). Allylic sites are where a methylene (CH_2) group is located adjacent to one double bond. They are highly susceptible to oxidation, but bis-allylic sites are even more susceptible due to the location of the methylene group between two double bonds (Rocha Jr et al., 2019). Given the lack of PUFAs in the *Synechococcus* strains, predicted OS values of zero (Table 3) are surprising. The BiodieselAnalyzer© tool (Talebi et al., 2014) considers both APE and BAPE values in calculating OS according to Knothe (2002). BAPE values were zero for the strains which did not contain PUFAs, indicating high oxidative stability

(Knothe, 2002). In order to determine whether OS values could be changed by entering non-zero data, a 0.1% FAME content was entered for C18:2 and C18:3 PUFAs. Without noticeably affecting other outputs, this small change increased the OS of the *Synechococcus* strains to almost 600 h, clearly meeting the EN 14214 requirement of >8 h. Thus, the low BAPE level fits with unsaturation data in predicting a (very) high OS, which can only be extracted from the BiodieselAnalyzer© tool when non-zero amounts of bis-allylic components are used as input. This highlights the risk of obscured or misinterpreted data coming from unique organisms, such as these Nordic *Synechococcus* strains, which do not fit the assumptions of the model. Here, it should be noted that of the genus, only freshwater *Synechococcus* strains which are classified as Group 1 under the Kenyon-Murata system (as described in Los & Mironov, 2015) would be expected to exhibit a PUFA-free FAME profile.

Cold-flow properties (represented by CFPP) are generally considered to be poor where oils contain high concentrations of SFAs, resulting in increased melting temperature range of the fuel (Stansell et al., 2012). As climate varies between countries in the European Union, so does the CFPP requirement. The *Synechococcus* strains were predicted to be most suited to cold weather, with the lowest CFPP values (Table 3). This is somewhat surprising as these strains also contained the largest SFA abundance (~50% relative abundance, Table 2). However, SFA chain length also affects melting points and here *Synechococcus* strains are clearly differentiated by their C14:0 content, comprising almost half of SFA totals. In contrast, the SFA contents of the other strains consisted almost wholly of C16:0, contributing to their higher CFPP values. It is possible, however for CFPP to alter over the course of a specific growth period. Strain UHCC0027, evaluated in a separate pilot scale study (Jämsä et al., 2017), showed a large increase in CFPP as the wastewater medium became depleted in ammoniacal Nitrogen and the algal cells entered stationary phase. Driving the change in CFPP was increases in C16:0, C18:0, C18:1 FAs and decreases in all PUFAs. In the same study, it was found that an experiment run in parallel at a colder temperature produced slightly better cold flow properties on the basis of CFPP, likely due to the unsaturation of cell membranes required for cold tolerance (Murata et al., 1992). Thus, it is possible to 'tune' the cold performance properties of a fuel on the basis of growth conditions.

There are many workarounds available to improve the performance of biodiesel fuels. These can generally involve additives such as antioxidants for oxidative stability, blending with other fuels and/or improvements of raw materials. Improvements in oxidative stability may also be achieved via control of unsaturation through knock outs of specific desaturases (refer Sarsekeyeva et al., 2015 and references within).

4.4 | Nordic cyanobacterial strains contain abundant C18 PUFAs for nutraceutical application

Whilst there are many promising approaches to improving cyanobacterial and algal biodiesel, its industrial viability has yet to be fully realised. Meanwhile, industrial scale production of photosynthetic microbe based nutraceuticals and cosmetics is decades old (Ratledge, 2013). In contrast to biodiesel, the nutraceutical industry is built mainly around extracting an abundance of PUFAs. PUFAs are highly valuable due to their anti-inflammatory action effecting a variety of clinically challenging conditions such as: cardiovascular disease, diabetes, cancer, Alzheimer's disease, dementia, depression, visual and neurological development, and maternal and child health (Shahidi & Ambigaipalan, 2018). DHA, in particular, is known to be essential for infant brain function and development, for normal brain function in adults and to be protective against Alzheimer's disease, type II diabetes and coronary heart disease (Horrocks & Yeo, 1999). The native *Synechococcus* strains, demonstrating the best biodiesel qualities due to a lack of PUFA content, thus show the least potential for employment in nutraceutical production. However, with the exception of the *Synechococcus* strains, all cyanobacterial and algal strains contained large abundances of PUFAs (38–53%, Table 2). Linolenic acid (C18:3) was the main contributor to PUFA abundances across all PUFA containing strains, except *Calothrix* (Table 2). The majority of linolenic acid was omega-6 gamma-linolenic acid (GLA, C18:3 n6) which ranged in content from 13 to 21% of total FAs in the cyanobacteria strains except for *Synechococcus* and *Calothrix*. GLA is found in human milk and botanical seed oils and has been demonstrated in numerous in vitro and in vivo animal models to attenuate inflammatory responses (Sergeant et al., 2016). As well as GLA, omega-3 alpha-linolenic acid (ALA, C18:3 n 3) was also present, predominantly in green algae and *Calothrix* strains (ranging between 17 and 30%). ALA is nutritionally important as it cannot be directly synthesised by humans endogenously, making it one of two 'essential' fatty acids (Lordan et al., 2020). The other 'essential' human fatty acid is linoleic acid (LA, C18:2 n6), which at 21% relative abundance, was the predominant PUFA for the native filamentous cyanobacteria *Calothrix* sp. 336/3 (Table 2). LA has long been purported to be beneficial in preventing cardiovascular disease. Indeed, a recent meta-analysis of prospective cohort studies found higher linoleic intake to be significantly associated with a modestly lower risk of mortality from all causes, cardiovascular disease, and cancer (Li et al., 2020).

Overall, this work has demonstrated the potential of Nordic cyanobacterial lipids as raw materials in the blue bioeconomy and presented valuable assessments applicable to this and other photosynthetic microbial culture collections in regard to lipid-based fuel and bio-industries. This work builds on previous assessments of Nordic photosynthetic microbes for lipid accumulation in wastewater at lab- (Lynch et al., 2015) and pilot scale (Jämsä et al., 2017), towards integrated, closed-loop production platforms. We anticipate that improvements in genetic engineering approaches to TAG accumulation and environmentally tailored FAME profiles will play important roles in moving such platforms forward in the near future. It is our hope that this work will inform future approaches to understanding and developing the photosynthetic microbial resources available in the Nordic region.

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AUTHOR CONTRIBUTIONS

F.L. and Y.A. conceived the study, F. L., A. S-S., Y. A. designed the experiments; A. S -S. performed the experiments. F. L., A. S-S. and S. Ş. analysed the data. F. L. took the lead in writing the manuscript. The manuscript was revised and approved by all authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Fiona Lynch https://orcid.org/0000-0002-0443-3511
Yagut Allahverdiyeva https://orcid.org/0000-0002-9262-1757

REFERENCES

Aizouq, M., Peisker, H., Gutbrod, K., Melzer, M., Hölzl, G. & Dörmann, P. (2020) Triacylglycerol and phytyl ester synthesis in *Synechocystis* sp. PCC6803. *PNAS*, 117, 6216–6222.

Cheregi, O., Ekendahl, S., Engelbrektsson, J., Strömberg, N., Godhe, A. & Spetea, C. (2019) Microalgae biotechnology in Nordic countries–the potential of local strains. *Physiologia Plantarum*, 166, 438–450.

Deshmukh, S., Bala, K. & Kumar, R. (2019) Selection of microalgae species based on their lipid content, fatty acid profile and apparent fuel properties for biodiesel production. *Environmental Science and Pollution Research*, 26, 24462–24473.

Edwards, M.R., Berns, D.S., Ghiorse, W.C. & Holt, S.C. (1968) Ultrastructure of thermophilic blue green alga *Synechococcus lividus* Copeland. *Journal of Phycology*, 4, 283–298.

Ferro, L., Hu, Y.O., Gentili, F.G., Andersson, A.F. & Funk, C. (2020) DNA metabarcoding reveals microbial community dynamics in a microalgae-based municipal wastewater treatment open photobioreactor. *Algal Research*, 51, 102043.

Hammer, Ø., Harper, D.A. & Ryan, P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 9.

Hazel, J.R. (1995) Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annual Review of Physiology*, 57, 19–42.

Horrocks, L.A. & Yeo, Y.K. (1999) Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research*, 40, 211–225.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. et al. (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal*, 54, 621–639.

Isojärvi, J., Shunmugam, S., Sivonen, K., Allahverdiyeva, Y., Aro, E.M. & Battchikova, N. (2015) Draft genome sequence of *Calothrix* strain 336/3, a novel H_2-producing cyanobacterium isolated from a Finnish lake. *Genome Announcements*, 3, e01474–e01414.

Jämsä, M., Lynch, F., Santana-Sánchez, A., Laaksonen, P., Zaitsev, G., Solovchenko, A. et al. (2017) Nutrient removal and biodiesel feedstock potential of green alga UHCC00027 grown in municipal wastewater under Nordic conditions. *Algal Research*, 26, 65–73.

Kenyon, C.N. (1972) Fatty acid composition of unicellular strains of blue-green algae. *Journal of Bacteriology*, 109, 827–834.

Kenyon, C.N., Rippka, R. & Stanier, R.Y. (1972) Fatty acid composition and physiological properties of some filamentous blue-green algae. *Archiv für Mikrobiologie*, 83, 216–236.

Knothe, G. (2002) Structure indices in FA chemistry. How relevant is the iodine value? *Journal of the American Oil Chemists' Society*, 79, 847–854.

Knothe, G. (2009) Improving biodiesel fuel properties by modifying fatty ester composition. *Energy & Environmental Science*, 2, 759–766.

Knothe, G. (2012) Historical perspectives on biofuels. In: Sayigh, A. (Ed.) *Comprehensive renewable energy*, Vol. 5, 1st edition. Amsterdam: Elsevier B.V., pp. 11–14.

Komárek, J. (2018) Several problems of the polyphasic approach in the modern cyanobacterial system. *Hydrobiologia*, 811, 7–17.

Lang, I., Hodac, L., Friedl, T. & Feussner, I. (2011) Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. *BMC Plant Biology*, 11, 124.

Li, J., Guasch-Ferré, M., Li, Y. & Hu, F.B. (2020) Dietary intake and biomarkers of linoleic acid and mortality: systematic review and meta-analysis of prospective cohort studies. *The American Journal of Clinical Nutrition*, 112, 150–167.

Lordan, R., Redfern, S., Tsoupras, A., Zabetaki, I. & Ioannis. (2020) Inflammation and cardiovascular disease: are marine phospholipids the answer? *Food & Function*, 11, 2861–2885.

Los, D.A. & Mironov, K.S. (2015) Modes of fatty acid desaturation in cyanobacteria: an update. *Life*, 5, 554–567.

Los, D.A. & Murata, N. (2004) Membrane fluidity and its roles in the perception of environmental signals. *Biochimica et Biophysica Acta*, 1666, 142–157.

Los, D.A., Mironov, K.S. & Allakhverdiev, S.I. (2013) Regulatory role of membrane fluidity in gene expression and physiological functions. *Photosynthesis Research*, 116, 489–509.

Lynch, F., Santana-Sánchez, A., Jämsä, M., Sivonen, K., Aro, E.M. & Allahverdiyeva, Y. (2015) Screening native isolates of cyanobacteria and a green alga for integrated wastewater treatment, biomass accumulation and neutral lipid production. *Algal Research*, 11, 411–420.

van de Meene, A.M., Hohmann-Marriott, M.F., Vermaas, W.F. & Roberson, R.W. (2006) The three-dimensional structure of the cyanobacterium *Synechocystis* sp. PCC 6803. *Archives of Microbiology*, 184, 259–270.

Murata, N., Wada, H. & Gombos, Z. (1992) Modes of fatty-acid desaturation in cyanobacteria. *Plant and Cell Physiology*, 33, 933–941.

Peramuna, A. & Summers, M.L. (2014) Composition and occurrence of lipid droplets in the cyanobacterium *Nostoc punctiforme*. *Archives of Microbiology*, 196, 881–890.

Ratledge, C. (2004) Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie*, 86, 807–815.

Ratledge, C. (2013) Microbial production of polyunsaturated fatty acids as nutraceuticals. In: *Microbial production of food ingredients, enzymes and nutraceuticals*. United Kingdom: Woodhead Publishing, pp. 531–558.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stainer, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cynobacteria. *Microbiology*, 111, 1–61.

Rocha, J.G., Jr., dos Santos, M.D., Madeira, F.B., Rocha, S.F., Bauerfeldt, G. F., da Silva, W.L. et al. (2019) Influence of fatty acid methyl ester composition, acid value, and water content on metallic copper corrosion caused by biodiesel. *Journal of the Brazilian Chemical Society*, 30, 1751–1761.

Ros, E. (2020) Linoleic acid intake and reduction in mortality: the icing on the cake of health benefits from n-6 PUFAs? *The American Journal of Clinical Nutrition*, 112, 3–4.

Ryckebosh, E., Muylaert, K. & Foubert, I. (2012) Optimisation of an analytical procedure for extraction of lipids from microalgae. *Journal of the American Oil Chemists' Society*, 89, 189–198.

Sahu, A., Pancha, I., Jain, D., Paliwal, C., Ghosh, T., Patidar, S. et al. (2013) Fatty acids as biomarkers of microalgae. *Phytochemistry*, 89, 53–58.

Salazar, V.W., Tschoeke DA Swings, J., Cosenza, C.A., Mattoso, M., Thompson, C.C. & Thompson, F.L. (2020) A new genomic taxonomy system for the *Synechococcus* collective. *Environmental Microbiology*, 22, 4557–4570.

Sarsekeyeva, F., Zayadan, B.K., Usserbaeva, A., Bedbenov, V.S., Sinetova, M.A. & Los, D.A. (2015) Cyanofuels: biofuels from cyanobacteria. Reality and perspectives. *Photosynthesis research*, 125, 329–340.

Schulze, P.S., Hulatt, C.J., Morales-Sánchez, D., Wijffels, R.H. & Kiron, V. (2019) Fatty acids and proteins from marine cold adapted microalgae for biotechnology. *Algal Research*, 42, 101604.

Sergeant, S., Rahbar, E. & Chilton, F.H. (2016) Gamma-linolenic acid, dihommo-gamma linolenic, eicosanoids and inflammatory processes. *European Journal of Pharmacology*, 785, 77–86.

Shahidi, F. & Ambigaipalan, P. (2018) Omega-3 polyunsaturated fatty acids and their health benefits. *Annual Review of Food Science and Technology*, 9, 345–381.

Sheehan, J., Dunahay, T., Benemann, J. & Roessler, P.G. (1998) US Department of Energy's Office of Fuels Development, July 1998. In: *A look back at the US Department of Energy's Aquatic Species Program – biodiesel from algae, close out report TP-580-24190*. Golden, CO: National Renewable Energy LAboratory.

Shi, J., Podola, B. & Melkonian, M. (2007) Removal of nitrogen and phosphorus from wastewater using microalgae immobilised on twin layers: an experimental study. *Journal of Applied Phycology*, 19, 417–423.

Sioen, I., van Lieshout, L., Eilander, A., Fleith, M., Lohner, S., Szommer, A. et al. (2017) Systematic review on n-3 and n-6 polyunsaturated fatty acid intake in European countries in light of the current recommendations-focus on specific population groups. *Annals of Nutrition and Metabolism*, 70, 39–50.

Stamenković, M., Steinwall, E., Nilsson, A.K. & Wulff, A. (2020) Fatty acids as chemotaxonomic and ecophysiological traits in green microalgae (desmids, Zygnematophyceae, Streptophyta): a discriminant analysis approach. *Phytochemistry*, 170, 112200.

Stansell, G.R., Gray, V.M. & Sym, S.D. (2012) Microalgal fatty acid composition: implications for biodiesel quality. *Journal of Applied Phycology*, 24, 791–801.

Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A.W., Ojala, A. & Brett, M.T. (2013) Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology*, 71, 165–178.

Talebi, A.F., Tabatabaei, M. & Chisti, Y. (2014) BiodieselAnalyzer: a user-friendly software for predicting the properties of prospective biodiesel. *Biofuel Research Journal*, 1, 55–57.

Temina, M., Rezankova, H., Rezanka, T. & Dembitsky, V.M. (2007) Diversity of the fatty acids of the *Nostoc* species and their statistical analysis. *Microbiological Research*, 162, 308–321.

Van Wychen S, Ramirez K, Laurens LM (2016) Determination of total lipids as fatty acid methyl esters (FAME) by in situ transesterification: laboratory analytical procedure (LAP) (No. NREL/TP-5100-60958). National Renewable Energy Lab.(NREL), Golden, CO.

Wahlen, B.D., Willis, R.M. & Seefeldt, L.C. (2011) Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology*, 102, 2724–2730.

Wolk, C.P. (1973) Physiology and cytological chemistry of blue-green algae. *Bacteriology Reviews*, 37, 32–101.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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