Revision of *Coelastrella* (Scenedesmaceae, Chlorophyta) and first register of this green coccoid microalgae for continental Norway

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Abstract

A terrestrial green microalga was isolated at Ås, in Akershus County, Norway. The strain corresponded to a coccoid chlorophyte. Morphological characteristics by light and electron microscopy, in conjunction with DNA amplification and sequencing of the 18S rDNA gene and ITS sequences, were used to identify the microalgae. The characteristics agree with those of the genus *Coelastrella* defined by Chodat, and formed a sister group with the recently described *C. thermophila* var. *globulina*. *Coelastrella* is a relatively small numbered genus that has not been observed in continental Norway before; there are no previous cultures available in collections of Norwegian strains. Gas chromatography analyses of the FAME-derivatives showed a high percentage of polyunsaturated fatty acids (44–45%) especially linolenic acid (C18:3n3; 30–34%). After the stationary phase, the cultures were able to accumulate several carotenoids as neoxanthin, pheophytin a, astaxanthin, canthaxanthin, lutein, and violaxanthin. Due to the scarcity of visual characters suitable for diagnostic purposes and the lack of DNA sequence information, there is a high possibility that species of this genus have been neglected in local environmental studies, even though it showed interesting properties for algal biotechnology.

Keywords 18S rDNA · ITS · Algae phylogeny · Fatty acids · Electron microscopy · Strain FGS-001

Introduction

Chlorophyta are an ancient and taxonomically diverse lineage of green algae with approximately 8000 described species and an estimated of 5000 still undescribed species (Hadi et al. 2016). For a long time, the classification of these organisms has been entirely based on morphological and cytological features of vegetative stages in their life cycle (Darienko et al. 2015). Unfortunately, the identification of coccoid green algae often presents a challenge for algal taxonomists due to the scarcity of visual characters suitable for diagnostic purposes and the lack of DNA sequence information (Škaloud et al. 2016).

Traditionally, the order Chlorococcales sensu lato grouped the coccoid taxa and represented one of the most diverse groups of photoautotrophic cryptogams. However, later studies have managed to transfer many taxa to other orders or classes i.e. Chlorophyceae, Trebouxiophyceae, and Prasinophyceae (Krienitz and Bock 2012). Moreover, phenotypic plasticity and the presence of cryptic species have contributed to taxonomy complication, resulting in constant reassignments in these microalgae (Eliáš et al. 2010; Malavasi et al. 2016).

Members of the subfamily Coelastroideae have been previously placed in the families Oocystaceae, Chlorellaceae, and Scotielloideae based on shape, reproduction, cell wall morphology and composition (Kalina and Punčochářová 1987), until phylogenetic molecular studies were applied. Nowadays, DNA amplification and sequencing of the 18S rDNA and internal transcribed spacer (ITS) studies placed Coelastroideae within the family Scenedesmaceae, order Sphaeropleales (Hanagata 1998, 2001; Hegewald and Hanagata 2000, 2002; Hegewald et al. 2010; Kaufnerová and Eliáš 2013; Lee et al. 2016; Ancona-Canché et al. 2017).
According to AlgaeBase (Guiry and Guiry 2020), the subfamily Coelastroideae comprises seven genera: Coelastrella Chodat 1922 (16 species flagged as accepted taxonomically), Scotiellopsis Vinatzer 1975 (1 sp.), Asterarcys Comas Gonzales 1981 (1 sp.), Harioitina Dangeard 1889 (2 spp.), Dimorphococcus Braun 1855 (3 spp.), Coelastrum Nägeli 1849 (30 accepted species), and Graesiella Kalina and PunČochářová (1987) (1 sp.).

The genus Coelastrella has been known for almost 100 years and it is relatively small numbered. Coelastrella spp. are coccoid, elliptical until citiform. They occur as unicellular microalgae or in few-celled aggregations. These species are peculiar by their sculptured cell wall with 16–40 meridional ribs with or without polar thickenings (Uzunov et al. 2008; Kaufnerová and Eliáš 2013). Ultrastructurally, cells are uninucleate, present numerous conspicuous vacuoles, a single cup-shaped and parietal chloroplast, each with one pyrenoid surrounded by 2(3) starch plates. The cell wall is double layered, with an inner cellulose component and an outer trilaminar one where acetolysis-resistant material (spongopollenin) resides (Tschaikner et al. 2007a, 2008). Asexual reproduction occurs by 2–16 auspores released by rupture of parental cell wall (Guiry and Guiry 2020). Together with other morphological features like cell form, chloroplast and pyrenoid structures, the characteristic differences in the wall sculptures are useful for identification of the species (Gärtner and Ingolić 1993; Hanagata et al. 1996; Tschaikner et al. 2007a, b). The type species of the genus is Coelastrella striolata Chodat 1922.

Recently, diverse studies have reported the biotechnological interest of this genus due to pigments and fatty acid content, as well as for a potential use for bioremediation (Abe et al. 2007; Hu et al. 2013; Dimitrova et al. 2017; Kawasaki et al. 2013; Luo et al. 2016; Thao et al. 2017; Wang et al. 2019b). In the last decades, the biotechnological use of microalgae has raised the interest from the industry (Wijnfels et al. 2010). Microalgae have been found to be the most promising feedstock in terms of their biomass productivity, high oil content, strong adaptive capacity to adverse environments, heavy metals, toxicants, high CO2 concentration and no competition with cultivable land (Chisti 2007). The screening of native algal species and strains is necessary for assessing the biotechnological potential of microalgae, especially in harsh environments; some of those species may be more beneficial than the commercially available strains.

In the present study, we report a terrestrial coccoid green microalga FGS-001, isolated at As, in Akershus County, Norway, which was identified as a Coelastrella strain, until now not described for continental Norway. Furthermore, very little is known about the distribution of this genus worldwide. The objectives of the study were (1) to increase knowledge on terrestrial Norwegian microalgae, (2) to characterize the isolated strain, (3) to determine its pigment and fatty acid composition profile, (4) to determine the phylogenetic relationships with known algal strains.

Materials and methods

Microorganism isolation, medium and culture conditions

The strain FGS-001 was isolated from a foliose, land-living colony of Nostoc commune, known locally as «glye» in Norwegian language. A sample of the cyanobacterium colony was collected in summer 2016 outside the Center for Climate Regulated Plant Research (SKP), Norwegian University of Life Sciences, Campus As, Akershus County, South East Norway (N 59° 40′ 5.81292″, E 10° 46′ 14.92156″ (EU89)). The sample was washed with sterile Milli-Q water, exposed to light and enriched nutrient solution (a modified Kristalon Indigo medium) without aeration in a 50 mL conical flask.

Initially, only cyanobacteria belonging to Nostocales were visible. After 3–4 weeks of culture, green microalgae were observed under light microscope, growing inside fragments of the colony. Further isolation of the microalga was achieved by consecutive transfers to fresh medium.

Kristalon Indigo standard nutrient solution according to producer (Yara, Norway) is composed NO3− 7.5%; NH4+ 1%; P 4.9%; K 24.7%; Mg 4.2%; S 5.7%; B 0.004%; Cu 0.004%; Fe 0.2%; Mn 0.06%; Mo 0.004%, and Zn 0.027%. A concentration was used of 0.01 g per liter of milli-Q water, enriched with 0.01 g urea and 0.002 g Opti-P 0-20-0 (Yara, Norway). The microalga was able to grow in a Tris–acetate–phosphate (TAP) (–) acetate media (see Harris 1989), and modified Provasoli nutrient media (West and McBride 1999), as well.

Finally, the isolated strain was able to grow in tubular photobioreactors (250 mL). It was cultivated under continuous illumination at a surface incident irradiance of 175 µmol m−2/s at 20.0 ± 2 °C at constant aeration under filtered air flow containing 1 ± 0.2% CO2 (v/v), and at an initial pH of 7.0.

Dry matter was determined gravimetrically. Aliquots (2 mL) of samples were harvested from the photobioreactors in pre-weighed tubes by centrifugation. The supernatant was discarded, and pellets were dried at 105 °C until they reached a constant weight (Goecke et al. 2015). Growth curve was determined by linear regression of the natural log of cell biomass vs. time for the data plotted in Fig. 4.

A voucher specimen will be deposited in the Norwegian Culture Collection of Algae (NORCCA) (https://niva-cca.no/).
Optical microscopy

To obtain a detailed morphological characterization of cultured microalgae, we investigated it by different microscopical techniques.

Light microscopy observations were performed using a Leica DM5000B microscope (Leica CTR5000, Leica Microsystems Limited, Heerbrugg, Switzerland) equipped with the attached Leica camera (DC200), and microphotographs were processed with the Leica Application Suite v4.3 image program (LAS 4.3).

Additionally, to corroborate that the cells are uninucleated, samples were stained for 15 min with the nuclear marker DAPI (4',6-diamidino-2-phenylindole; 10 μg/mL stock; Sigma-Aldrich, Saint Louis Missouri, USA), and analyzed using a UV filter under fluorescence microscopy.

Transmission electron microscopy (TEM)

Samples from two different culture ages (exponential and stationary phase) were fixed for transmission electron microscopy using chemical fixation protocols according to Olsen et al. (2015). Briefly, cells were harvested, centrifuged and prefixed in 1.25% glutaraldehyde and 2% formaldehyde in phosphate-buffered saline (PBS) solution for a minimum of 24 h. After washing several times in PBS and cacodylate buffer (0.1 M, pH 7.2), the cells were post fixed for 1 h at room temperature in 1% osmium tetroxide (OsO4), and washed again in cacodylate buffer. After dehydration through an ethanol series (15 min in 70, 90, 96%, and 4 × 15 min in 100%), cells were infiltrated and embedded in LR-White resin (Electron Microscopy Sciences, USA), and thinned using a UV filter under fluorescence microscopy.

Scanning electron microscopy (SEM)

Algae were treated using the same fixing method described above for TEM. After, coverslips coated with 1 mg mL⁻¹ poly-L-lysine were placed to allow algae to settle for 20 min. The fixed algae culture for SEM examination were washed thoroughly in 0.1 M sodium cacodylate buffer (SCB) and dehydrated with 10 min steps in ascending ethanol series (50–100%) as in Wiik-Nielsen et al. (2016). The samples were processed in a BAL-TEC Critical Point Dryer CPD 030, (BAL-TEC AG Lichtenstein), and a thin conductive coating of gold–palladium was applied to the samples using a Polar on Sputter Coater SC7640 (Quorum Technologies, UK). The coated samples were mounted on aluminum stubs, examined and photographed with a Zeiss EVO-50-EP scanning electron microscope at an accelerating voltage of 20 kV in the secondary emission mode.

DNA amplification and sequencing

For this study, the nuclear 18S rDNA gene and ITS regions of the alga were sequenced as described below.

Cells from 1 mL of algal culture were harvested by centrifugation, resuspended in 50 μL of EDTA 10 mM ph 8, and incubated at 100 °C for 10 min. Then, samples were cooled down at 4 °C for 10 min and resuspended by vortex. Finally, after 1 min of centrifugation at 10,000 rpm, 1 μL of supernatant was used to perform the PCR reactions.

The primer sets HET F (5′-ACCTGGTGTGATCCTGCA GTAGTCATAC-3′) and HET R (5′-GGTTACACTGCG AAAACCTTGTACGACTTCA-3′) (Cavalier-Smith and Chao 2006), were used for the amplifications of the 18S rDNA regions.

The primer sets ITS1 (5′-TCCGTAGGTAACCTGCG G-3′)/ITS4 (5′-TCCTCGGTATTGATATGC-3′) (White et al. 1990), were used for the amplifications of the ITS regions.

All fragments were amplified with Q5 High-Fidelity DNA Polymerase (New England BioLabs).

Purified and concentrated PCR products were used as template for Sanger dideoxy sequencing at GATC Services (Eurofins, Germany), using the same primers sets employed in the amplification of the fragments, and 18F2 (5′-GCT CGTAGTTGGATTTCTGG-3′) (this study), in the case of 18S rDNA regions.

New sequences generated by this investigation were submitted to the nucleotide database GenBank from the National Center for Biotechnology Information (NCBI), with GenBank accession numbers MK064224 and MK040329.

Phylogenetic analysis

The identity of the isolates was assessed by a phylogenetic analysis using BEAST v1.10.4 (Suchard et al. 2018). Sequences for 18S rRNA and internal transcribed spacer (ITS) were taken from Wang et al. (2019b) and downloaded from NCBI’s GenBank (NCBI Resource Coordinators 2017) database using the respective accession numbers. Nucleotide sequences generated in this study were added to the data set, and a multiple sequence alignment was computed with MAFFT v7.310 using the L-INS-i strategy (Katoh et al. 2002; Katoh and Standley 2013). To remove uninformative sites and reduce the matrix to the loci of interest, the alignment was trimmed with trimAl (Capella-Gutiérrez et al. 2009) with the parameters -gt 0.8 -st 0.001 -cons 0.6. After manual inspection and adjustment of the alignment, the best nucleotide substitution model was determined based
on AICc calculations by the modelTest function from the R package phangorn v2.5.3 (Schliep 2011; Darriba et al. 2012) using R v3.6.0 (R Core Team 2019). Thus, nucleotide substitutions in the 18S sequences were approximated with the GTR + Γ + I model (Hasegawa et al. 1985; Tavaré 1986; Yang 1994). For the ITS sequences, the GTR + Γ substitution model was used (Hasegawa et al. 1985; Tavaré 1986). Heterogeneity of the substitution rate was approximated by four discrete Γ categories in both cases. Phylogenetic trees were inferred assuming an uncorrelated relaxed molecular clock prior following a log-normal distribution (Drummond et al. 2006), assuming a Yule speciation process (Yule 1925; Yang 1994). For the ITS sequences, the GTR + Γ substitution model was used (Hasegawa et al. 1985; Tavaré 1986).

Analysis of pigment composition

Aliquots of 25–40 mg of freeze-dried algae material were weighed into lysis tubes (Type C, Analytik Jena, Jena, Germany) and 500 µL of ethanol (gradient grade, Merck, Darmstadt, Germany) were added. Cells were mechanically broken in a swing mill (MM 2000, Retsch, Haan, Germany) for 3 min. Afterwards the cells were centrifuged at 3000 rcf (5415R, Eppendorf, Hamburg, Germany) and the supernatant was recovered. This procedure was repeated twice until the supernatant was colorless. The combined extracts were dried under nitrogen at 40 °C in an evaporator (EVA-EC1 with metal block thermostat EC-1 V-130, both VLM, Bielefeld, Germany) and afterwards resuspended in a defined volume of ethanol and filtered through a 0.45 µm membrane filter (Chromafil Xtra PET -45/25, Macherey–Nagel, Düren, Germany). The samples were measured in an Ultra-performance liquid chromatography mass spectrometry (UPLC-MS), coupled to photodiode array detection (PDA), a UPLC-PDA-MS system (Waters, Milford, USA) with a Corteces C18 column (2.7 µm, 90 Å, 3 x 100 mm, Waters, Milford, USA), with a gradient of Millipore water and acetonitrile (hypergrade for LC–MS, Merck, Darmstadt, Germany) both acidified with 0.01% formic acid (99% ULC/MS, Biosolve B.V., Valkenswaard, Netherlands). Starting conditions were 70% water, decreasing to 10% after 4 min. These conditions were kept stable further 10 min. Afterwards a washing step with 70% water was attached for 4 min. The flow velocity was 0.5 mL/min continuously. Column temperature was set at 40 °C and the spectra were measured via a PDA (2998 PDA Detector, Waters, Milford, USA) in a range of 200 to 800 nm. The mass spectrometer with electrospray ionization (ESI) (Acquity QDA, Waters, Milford, USA) was operated in positive mode with a cone voltage of 15 V and a probe temperature of 600 °C, measuring in a range of 150 to 1250 m/z.

As references standards Astaxanthin (≥97% from Blakeslea trispora, Sigma-Aldrich, St. Louis, USA) and Lutein (pharmaceutical secondary standard, Sigma-Aldrich, St. Louis, USA) were used. Further carotenoids were determined by comparison with literature.

For the photometric measurements (for full spectrum for chlorophyll detection), samples were prepared similarly in quantities of 5 to 10 mg, extracted in ethanol (≥99.5%, Ph.Eur., Carl Roth, Karlsruhe, Germany), but measured in different concentrations in a multiwellplate (96F, TPP Techno Plastic Products AG, Trasadingen, Swiss) in an microplate reader (Infinite M Plex, Tecan, Männedorf, Swiss) or alternatively in a photometer (DR 6000, Hach Lange, Düsseldorf, Germany) with a spectrum from 300 to 800 nm in steps of 2–5 nm. Due to biomass limitations, pigments were detected although not quantified.

Analysis of fatty acid composition

Conversion and extraction of algal lipids to fatty acid methyl esters (FAME) was done by the method of O’Fallon et al. (2007) with a minor modification concerning the volumes. Samples (0.3 g of freeze-dried sample) were placed into Pyrex culture tubes to which 0.3 mL of the C13:0 internal standard (0.5 mg of C13:0/mL of MeOH), 0.56 mL of 10 M KOH in water, and 4.24 mL of MeOH were added. The tubes were incubated in a 55 °C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.46 mL of 24 M H2SO4 in water was added. The tube was mixed by inversion and with precipitated K2SO4 present was incubated again in a 55 °C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate. The fatty acid composition of the FAME was determined by capillary GC on a RT-2560, 100 m x 0.25 mm x 0.20 µm capillary column (Restek) installed on a Trace GC ULTRA gas chromatograph equipped with a Triplus autosampler with PTV inlet and a flame ionization detector, and controlled by Chromleleon 7.2 Chromatography Data System.
The initial oven temperature was 140 °C, held for 5 min, subsequently increased to 240 °C at a rate of 4 °C/min, and then held for 20 min. Helium was used as the carrier gas at a flow rate of 2 mL/min, and the column head pressure was 270 kPa. Both the injector and the detector were set at 250 °C. The injector was set in split mode and the split ratio was 20:1. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards (Supelco standard FAME mixture) described previously.

**Results**

**Light microscopy**

The isolated strain FGS-001 is a unicellular dark green microalga, although some cells were in aggregation. It grows in variable forms from globose to spheroidal to ellipsoidal (Fig. 1a). The cells presented variable sizes, but usually were (6–)7–10(–13) µm long (with an average of 8.42 ± 1.34 µm) and (4.5–)6–9(–11) µm wide (with an average of 7.14 ± 1.39 µm) (Fig. 1). Cell wall appears to be hyaline, and a cup-shaped chloroplast was easy to observe. A stricken and single pyrenoid was clearly visible in the vegetative cells as well as in autospores (Fig. 1a, b).

Meridional ribs were very difficult to observe under light microscopy. Although a smooth, apical thickening was visible in few cells (Fig. 1b).

Asexual reproduction takes place by autospores formed by successive bipartition of the protoplast to produce 4 to 16 spores within the mother cell (Fig. 1c). Autospores are elongated and polar thickenings were easier to notice, as well as their pyrenoids. They are discharged through rupture of the cell wall of the sporangium although some remain surrounded by sporangium walls (Fig. 1b, c). No sexual reproduction was observed.

**Electron microscopy.**

Transmission electron microphotographs of a group of vegetative and reproductive cells of *Coelastrella* sp. FGS-001 is shown in Fig. 2. Figure 2a is an example of the variable forms, from globose to ellipsoidal cells, which were found with variable sizes. The distribution of cell organelles is showed in detail by this technique. Vegetative cells presented a thin cell wall, where very smooth ribs were also visible at the cell wall surface (Fig. 2b). Internally, the single chloroplast proliferates throughout the cell with dense thylakoids; it presents a large cup-shaped form, which is associated with a single, prominent, spheroidal, and central pyrenoid structure with a ring of starch plates (n = 2) surrounding it, clearly

![Fig. 1 Unialgal culture of the strain FGS-001.](image)
The single nucleus was located next to the pyrenoid (Fig. 2c). Occasionally, a second pyrenoid was observed by cell division (not shown).

The autospores presented a slightly smaller size than the vegetative cells, with a single nucleus, several vacuoles, lipid/starch droplets, thicker starch plates surrounding the pyrenoid, and a thick and irregular cell wall, with clear ribs of different sizes at the surface level (Fig. 2c). A variable number of autospores were visible at the autosporangia (Fig. 2d).

The cell wall of Coelastrella sp. FGS-001 consists of multiple layers, with an inner cellulose component and an outer apparently trilaminar one (Fig. 2e).

Scanning electron microscopy was performed at different times (4–18 days) of a living culture of the strain FGS-001. Different cell morphologies were observed ranging from globose, spheroidal until ellipsoidal. As shown in the micrographs (Fig. 3), the unicellular organism presents characteristic cell wall sculptures in form of meridional ribs, although they were more clearly visible at autospores and at younger states of the cells. The number of ribs may vary accordingly, although the difference was not statistically quantified (mainly around 10–16, with a possible maximum of 20) (Fig. 3). These ribs converged at two poles of the cells, and a smooth polar thickening was formed (Fig. 3b).

Groups of autospores were discharged through a rupture of the cell wall of the sporangium which corresponded to the aggregations we observed under light microscopy (Fig. 3b, c).

### Growth characteristics

Coelastrella sp. FGS-001 was cultured in a nutrient rich media at 175 μmol/m²/s and 20.0 ± 2 °C temperature for 10 days to observe the growth pattern in batch culture. The alga grew well in the temperature range of 15–20 °C (data

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**Fig. 2** a Transmission electron microphotographs of a group of vegetative and reproductive cells of Coelastrella sp. FGS-001 isolated from Ås, Akershus, Norway. Scale bar of 10 µm. b Young vegetative cell at exponential growth. A cup-shaped chloroplast (Ch) with dense thylakoids, the associated pyrenoid (P) structure with starch plates, and a thin cell wall, are clearly visible on the strain. Very smooth ribs are also present at the cell wall surface. c Autospore showing the single nucleus (N), vacuoles (V), thick starch plates (St), lipid droplets (L) and a thick and irregular cell wall (CW), with clear ribs of different sizes. d Autosporangia showing the formation of autospores, six cells are visible. e Cell wall detail with an outer trilaminar component. Scale bar: a = 10 µm; b–d = 2 µm; and e = 0.5 µm
Dry matter was measured (Fig. 4). The stationary phase was observed from day 8 onwards, probably due to nutrient limitation in the media.

Phylogenetic associations

The nuclear 18S rDNA gene and ITS regions of the isolate FGS-001 were sequenced and compared to those of similar species in GenBank. As shown in Fig. 5, this new strain was grouped into one clade with other Coelastrella species and confirmed that the subfamily Coelastroideae are included in the monophyletic family Scenedesmaceae. We named the strain tentatively Coelastrella sp. FGS-001.

Coelastrella striolata (type species of the genus) and most of the available original culture strains seems to form a “core Coelastrella” group (Fig. 5). Our strain FGS-001 was placed within the “Coelastrella sensu lato” group, a sister group from the core Coelastrella group.

Pigment analysis

At the exponential phase, the main pigments of the strain FGS-001 were chlorophyll a and b, represented by a strong green color (Fig. 1b). After a few weeks at the stationary phase and without replacement of new nutrient media, the culture turned into yellowish-green until reddish-orange. This can be easily demonstrated on an agar plate at the exponential phase (Fig. 6a), and after a few weeks later of growth at the stationary phase (Fig. 6b). In the latter biomass, we were able to detect the presence of neoxanthin, pheophytin...
a, astaxanthin, canthaxanthin, lutein, an unknown carotenoid, and violaxanthin as the responsible pigments for this orange color (Fig. 6b, Table 2); although pigments were not quantified.

**Fatty acid results**

The fatty acid (FA) composition of freeze-dry biomass of the microalgal strain was analyzed using a gas
We were able to identify 17 fatty acids after 8 and 18 days of cultivation, which were composed of saturated and unsaturated FAs with 10 to 20 carbon atoms. The FA composition is represented in Table 3.

The major FAs in Coelastrella sp. FGS-001 (see Fig. 7) were C18:3 n3 (which ranged from 30.79 to 33.45% for 8 and 18 days cultivation, respectively); C18:1 n9c (22.40% to 23.33%); C16:0 (17.84% to 18.27%), C18:2 n6c (12.31% to 7.3%), and C16:1 (10.86% to 11.54%). It was found that the total amount of FAs in the algae cells was 90.2 to 96.9 mg/g after first and second sampling, from which 27.8 ± 6.5 to 32.4 ± 4.8 mg/g corresponded to linolenic acid, the main fatty acid on the microalga (Table 3, Fig. 7).

The average percentages of total polyunsaturated fatty acids (PUFAs) were the highest (45.5% to 44.1%), in comparison with total saturated fatty acids (SFAs; 18.9% to 19.3%) and total monounsaturated fatty acids (MUFAs; 35.5% to 36.6%) (Fig. 7, after 8 days cultivation).

Slight differences in the FA percentage were observed among 8 and 18 days of cultivation (Table 3). A slight increase was observed on the FAs C16:0; C16:1; C17:0; C18:1 n9c; C18:3 n6; C20:1; C18:3 n3; C20:2, and C22:1 n9, although less than 3%. C18:2 n6c was reduced in 5% after 18 days, and it was the most notorious change in the FA profile after 10 days difference.

Discussion

In the present study, we described a terrestrial green microalga isolated at Ås, Norway. The strain corresponded to a coccoid chlorophyte. Morphological characteristics by light, fluorescence and electron microscopy (using TEM and SEM), in conjunction with sequences of 18S rDNA and ITS region, were used to identify the strain. The characteristics agree with those of the genus Coelastrella defined by Chodat (Table 1), with a sister relationship with Coelastrella thermophila var. globulina recently described by Wang et al. (2019b). A list of all the accepted species of Coelastrella and their morphological features is displayed at Supplement Table 1.

To our knowledge, Coelastrella has not been registered in continental Norway before; thus, there were no previous cultures available on collections of Norwegian strains. We agree that due to the scarcity of visual characters suitable for diagnostic purposes (see Table 1) there is a high possibility that many species of coccoid microalgae (including Coelastrella spp.) have been neglected in local environmental studies (in Norway and elsewhere). Only the last year, four new species and two new varieties have been described of Coelastrella (Kawasaki et al. 2019; Wang et al. 2019b). In Norway, there are few observations of other related green coccoid microalgae, including Dimorphococcus and Coelastrum (also members of the Coelastroideae), and Chlorococcum spp. (see Artsdatabanken 2018, https://artsdatabanken.no/; Norwegian Culture Collection of Algae, NORCCA 2018; The Culture Collection of Cryophilic Algae, CCCryo 2018; Bruteig et al. 2001). Moreover, Kol (1963) described the species Scotiella norvegica from red snow of Finse, Hordaland County (west Norway), although a phylogenetic position of this species is lacking. Many fusiform snow algae inhabiting polar areas and high alpine zones were initially believed to be a member of this genus, but further studies have pointed several of the Scotiella ‘species’ as zygospores of chlamydomonad-like snow algae (Procházková et al. 2018; Remias et al. 2018; Table 1).

Further north, in the Svalbard archipelago, Kim et al. (2008) investigated snow algae from northwestern Spitsbergen and reported a Scotiellopsis sp. At the same area, another group of researchers collected Scotiella sp. from supraglacial sediments (Stibal et al. 2006), and Scotiella norvegica and Scotiellopsis terrestris from wet soil and moss samples (Matuła et al. 2007). Nevertheless, over the last years, several species of Scotiella and Scotiellopsis were transferred to other genera including Coelastrella (Kaufnerová and Eliáš 2013). Recently, in Svalbard, Coelastrella aeroterrestrica, Coelastrella rubescens, Coelastrella cf. rubescens, and a Coelastrella sp., were...
Table 1 Comparison of diacritical morphological features among “similar” genera of green coccoid microalgae, based on data recorded in AlgaeBase (Guiry and Guiry 2020) and original type strain species descriptions (Fas = flagged as accepted taxonomically by literature)

| Genus            | Organization                  | Cell walls (CW)                        | Chloroplast (CH), pyrenoid (Py) | Asexual reproduction | Phylogeny                                      |
|------------------|--------------------------------|----------------------------------------|---------------------------------|----------------------|-----------------------------------------------|
| Coelastrella     | solitary, temporary aggregated| 16–40 ribs, with AM                    | 1CH, parietal, cup-shaped; 1Py  | 2–16 autospores      | SphaO; SceF; CoeS                           |
| Chodat 1922, (16 Fas)* |                                 |                                        |                                 |                      | *polyphyletic                                  |
| Graesiella       | solitary                       | smooth CW, fine network of ribbing     | 1CH, parietal; 1Py              | 2–8–(16) autospores  | ChlaO incertae sedis                         |
| Kalina and PunČochařová 1987, (1 Fas) |                                 |                                        |                                 |                      | *many now as Chloromonas*                     |
| Scotella         | solitary                       | 6 defined longitudinal ribs            | –                               | –                    | (ChlaO; ChloF)                                |
| (Chodat) Fritsch 1912, (9 Fas) |                                 |                                        |                                 |                      | *soon replaced?                               |
| Scotiellopsis    | solitary, temporarily 2–4–(8)  | meridional ribs pole to pole, with AM  | 1CH, parietal, 1Py              | 2–(16) autospores    | ChlaO; SceF; CoeS                            |
| Vinatzer 1975, (1 Fas)* |                                 |                                        |                                 |                      |                                              |
| Asterarcys       | 2–4–8 celled coenobia embedded in mucilage | thin and smooth CW, with AM | 1CH, parietal cup-shaped; 1Py  | 4–8 autospores      | SphaO; SceF; CoeS                            |
| Comas Gonzales 1981, (1 Fas) |                                 |                                        |                                 |                      |                                              |
| Hariotina        | 4 celled coenobia              | –                                      | –                               | Daughter colonies    | SphaO; SceF; CoeS                            |
| Dangeard 1889, (2 Fas) |                                 |                                        |                                 |                      |                                              |
| Dimorphococcus   | colonies of 4-celled coenobia  | Smooth CW                              | 1CH, parietal; 1-3Py            | 4 autospores         | SphaO; SceF; CoeS                            |
| Braun 1855, (3 Fas) | with 16- or more- celled syncoenobia |                                        |                                 |                      |                                              |
| Coelastrum       | 4, 8, 16, 32 or 64 (-128) celled coenobia | smooth and wrinkled, specialized wall plaques | 1CH, parietal; 1Py          | daughter colonies    | SphaO; SceF; CoeS                            |
| Nägeli 1849, (30 Fas) |                                 |                                        |                                 |                      |                                              |
| Ematix           | 2–4–8 celled coenobia not embedded in mucilage | 3–6 longitudinal ribs, pole to pole | 1CH, parietal; 1Py              | 2–8 autospores     | ChlaO incertae sedis                         |
| Pascher 1943, (2 Fas)* |                                 |                                        |                                 |                      | *polyphyletic                                  |
| Ettlia           | solitary, temporary aggregated | Thin CW                                | 1CH, cup-shaped; 1Py            | 4–16–(64) zoospores; aplanospores | ChlaO incertae sedis |
| Komárek 1989, (7 Fas)* |                                 |                                        |                                 |                      | *valid genus?                                 |
| Chlorococcum     | solitary, temporarily aggregated | Smooth CW                              | 1CH, parietal, cup-shaped; 1Py or+ | Motile zoospores, with 2 flagella; aplanospores | ChlaO; ChloF |
| Meneghini 1842, (47 Fas)* |                                 |                                        |                                 |                      | *polyphyletic                                  |
| Tetracystis      | 4 celled coenobia              | –                                      | 1CH, parietal; 1Py              | 4–8 motile zoospores, with flagella | ChlaO; ChloF |
| Brown and Bold 1964, (15 Fas)* |                                 |                                        |                                 |                      | *type strain is now a Chlorococcum* |

The actual phylogeny is represented by Order Sphaeropleales (SphaO), Family Scenedesmaceae (SceF), and subfamilies Coelastroideae (CoeS), and Scenedesmoideae (SceS); and by the Order Chlamydomonadales (ChlaO), Families Chlamydomonadaceae (ChlaF), and Chlorococcaceae (ChloF).

AM sporopollenin or other acetoresistant material

*See comments on Phylogeny
identified by Borchhardt et al. (2017). However, apparently only strains of Chloromonas nivalis (ex Scotiella antarctica Fritsch, ex Scotiella nivalis (Chodat) Fritsch, ex Scotiella cryophila Chodat) are available from Svalbard in culture collections (see The Culture Collection of Cryptophic Algae, CCCCryo 2018). This makes us conclude that there are no previous cultures available on collections of Norwegian strains.

**Strain morphological identification**

The strain FGS-001 grew solitary, especially under aeration, and only small groups of cells were temporary aggregated, probably after autospores liberation. Without any aeration, the strain tended to form biofilm. Cells were also not embedded in mucilage. The family Scenedesmaceae contains numerous coenobial species of Desmodesmus, Neodesmus, and especially Scenedesmus, although some representatives of the latter genus are only known in solitary coccoid form. Other genera in this subfamily are either coenobial (e.g., Coelastrum, Hariotina) or solitary (e.g., Coelastrella, Scotiellopsis) (Fučíková et al. 2014). Thus, cell organization of daughter cells/autospores released by the sporangium is considered of taxonomic relevance as well, among these microalgae with related or “similar” green coccoid microalgae investigated with TEM see Pickett-Heaps and Staehelin (1975), Gopalakrishnan et al. (2014) or Shebanova et al. (2017) for Chloromonas spp., Chihara et al. (1994), Kawasaki et al. (2018), and Procházková et al. (2018), and Remias et al. (2018).

Using TEM, Gärtner and Ingolić (1993) and later Tschai kner et al. (2007b) showed a very similar liberation of daughter cells/autospores from an autosporangium from C. terrestris (strain N29 and strain SWK3:53 respectively, ex S. terrestris). Similar observations were made by PunČochářová and Kalina (1981) and Kaufnerová and Eliáš (2013) in C. rubescens CCA 475, Uzunov et al. (2008) in C. aeroterrestrica, and Kawasaki et al. (2019) in C. astaxanthina and Coelastrella sp. SAG 2123. The number of daughter cells/autospores released by the sporangium is considered of taxonomic relevance as well, among these microalgae (Table 1). We observed that apparently the strain FGS-001 liberated 2 to 6 autospores (Figs. 2c and 3b).

Transmission electron microscopy was useful to observe the inner structure and distribution of organelles on the cells. The characteristic complex cell wall was also seen with this method (Fig. 2e), a taxonomic characteristic of the genus (Table 1). The different ribs types were also observed with TEM (Fig. 2d). The chloroplast form position and the clear pyrenoid complex were recorded too and appears to be as described for Coelastrella (Fig. 2b). For comparison with related or “similar” green coccoid microalgae investigated with TEM see Pickett-Heaps and Staehelin (1975), Gopalakrishnan et al. (2014) or Shebanova et al. (2017) for Desmodesmus and Scenedesmus spp., Chihara et al. (1994), Hu et al. (1998) and Feng et al. (2016) for Chlorococcum, Cardon et al. (2018) for Enallaxis costatus and Acutodesmus spp., Eliáš et al. (2010) for Hylosedesmus, Matsuzaki et al. (2018), and Procházková et al. (2018), and Remias et al. (2018) for Chloromonas spp.

Pickett-Heaps and Staehelin (1975) showed the presence of other structures on the cell wall, like terminal spines, which is characteristic in many Scenedesmus species.
alga contained 16 and 18 carbon atoms identified by gas chromatography analyses. The main fatty acids of the A diverse array of fatty acids ranging from C10 until C20 were quantified which are absent in our isolate.

Cell wall surface, stigma, flagella and a posterior nucleus, 105), which is very useful for comparison with our strain. Interestingly, Miller (1978) presented TEM microphotographs of zoospores of Chlorococcum oleofaciens (Utxex 105), which is very useful for comparison with our strain. The authors showed the chlamydomonal-like structures, i.e. cell wall surface, stigma, flagella and a posterior nucleus, which are absent in our isolate.

Table 2 Pigment composition of biomass of Coelastrella sp. FGS-001 after the stationary phase

| Name                | Comment          |
|---------------------|------------------|
| Neoxanthin           | –                |
| Pheophytin a         | –                |
| Astaxanthin          | Both isomers     |
| Canthaxanthin        | Both isomers     |
| Lutein               | Both isomers     |
| Unknown carotenoid   | (m/z) appr. 468  |
| Violaxanthin         | –                |
| Chlorophyll a        | Major chlorophyll|
| Chlorophyll b        | –                |

Table 3 Detailed fatty acid composition of the isolated microalga FGS-001 under cultivation on day 8 and 18 (in mg/g; mean ± SD; n = 3)

| Fatty acid Name     | 8 days         | 18 days        |
|---------------------|----------------|----------------|
| C10:0               |                |                |
| Capric acid         | 0.027 ± 0.01   | 0.033 ± 0.01   |
| C12:0               | 0.370 ± 0.02   | 0.359 ± 0.04   |
| C14:0               | 0.140 ± 0.01   | 0.156 ± 0.01   |
| C16:0               | 16.083 ± 0.75  | 17.700 ± 2.60  |
| C16:1               | 9.790 ± 1.08   | 11.183 ± 1.30  |
| C17:0               | 0.177 ± 0.02   | 0.214 ± 0.05   |
| C17:1               | 1.539 ± 0.30   | 1.275 ± 0.13   |
| C18:0               | 0.305 ± 0.05   | 0.242 ± 0.03   |
| C18:1n9t            | 0.259 ± 0.14   | 0.107 ± 0.02   |
| Elaidic acid (trans)|                |                |
| C18:1n9e            | 20.191 ± 2.15  | 22.608 ± 2.74  |
| Oleic acid (cis)    |                |                |
| C18:2n6c            | 11.102 ± 3.94  | 7.127 ± 0.50   |
| Linoleic acid (cis) |                |                |
| C18:3n6             | 0.796 ± 0.08   | 0.894 ± 0.08   |
| Y-Linolenic acid    |                |                |
| C20:1               | 0.149 ± 0.01   | 0.214 ± 0.04   |
| Cis-11-Eicosenoic acid |            |                |
| C18:3n3             | 27.753 ± 6.55  | 32.406 ± 4.80  |
| Linolenic acid      |                |                |
| C20:2               | 1.365 ± 0.60   | 2.280 ± 0.40   |
| Cis-11,14-Eicosadienoic acid |      |                |
| C22:1n9             | 0.078 ± 0.01   | 0.074 ± 0.03   |
| Erucic acid         |                |                |
| C20:4n6             | 0.027 ± 0.00   | 0.021 ± 0.00   |
| Arachidonic acid    |                |                |
| FA total            | 90.15          | 96.89          |

A diverse array of fatty acids ranging from C10 until C20 were quantified by gas chromatography analyses. The main fatty acids of the alga contained 16 and 18 carbon atoms

Strain phylogeny

A faster and more accurate alternative for species identification and phylogenetic relationships among coccoid algal species is provided by DNA sequence comparisons (Škaloud et al. 2016). In general, our phylogenetic analysis of 18S rDNA and ITS region confirmed that the subfamily Coelastrideoideae are included in the monophyletic family Scenedesmaceae, although a more detailed analyses showed that the Coelastrella taxa belong to several different lineages within this family (Fig. 5). Our analyses did not clearly define a unique lineage for the Coelastrella taxa but add a new strain for such future elucidation. Similar conclusions were made by previous studies (see Hegewald et al. 2010; Kaufnerová and Eliáš 2013; Lee et al. 2016; Wang et al. 2019a).

Based on the 18S rDNA and ITS sequence phylogenetic tree phylogeny, we placed our strain into the order Sphaeropleales, as a sister order of the Chlamydomonadales, as observed previously (Hodač 2015; Watanabe and Lewis 2017; Fig. 5). Several morphological, ultrastructural and reproductive characteristics are shared (or appear to be) between these coccoid green microalgae (Table 1), therefore misidentification has been a problem for further phylogenetic studies. Several sequences available online carried misleading names and complicates the analysis of the group’s phylogeny (see Kaufnerová and Eliáš 2013; Lee et al. 2016). As mentioned, certain genera, as Scotiella, were found to be resistant stages (which presented a similar morphology to Coelastrideoideae: non-motile cysts with longitudinal ribs on cell surface) of other green algae as Chlamydomonadales i.e. Chloromonas spp. (Procházková et al. 2018; Remias et al. 2018). The genus are retained only for those species whose reproduction remain unknown (Hanagata 1998). Other genera have been separated from species (i.e. Scotiellopsis) by transferring them to other genera, based on new ultrastructural or molecular methods; remaining only few (old) species with unknown, too difficult to get, or none, biological material for further comparisons. Hence the importance of strain deposition on culture collections.

Similarly, as in Kaufnerová and Eliáš (2013), the type species of Coelastrella (C. striolata) and most of the available original (type culture) strains used for the description of several species of Coelastrella seems to form a “core Coelastrella” group (Fig. 5). Our strain FGS-001 grouped clearly into the subfamily Coelastrideoideae (family Scenedesmaceae) as well, specifically into the “Coelastrella sensu lato” group, a sister group from the core Coelastrella.

Further molecular phylogenetic investigations have raised concerns regarding the polyphyletic nature of some of those genera and further modifications are expected to happen as is the case of Chlorococcum (Kawasaki et al. 2015; Feng et al. 2016), Coelastrella (Kaufnerová and Eliáš 2013; Wang et al. 2019a), Coelastrum (Hegewald et al. 2010), and Ettlia (Pegg...
et al. 2015). Using 18 s rDNA sequences, Kaufnerová and Eliáš (2013), and later Wang et al. (2019a) demonstrated that the genus Coelastrella sensu lato is paraphyletic as species currently attributed to the genera Asterarcys, Scenedesmus or Ettlia are nested among taxa nominally representing Coelastrella. Clearly internal relationships among Coelastrella species are not solved yet and denser taxon sampling with more molecular markers is required to elucidate the classification of the strains (Lee et al. 2016). As mentioned by Wang et al. (2019b), it seems that 18S rDNA was too conserved to be used as a species-specific marker in this clade. We should clarify that a few original strains lacked available 18S sequences as Coelastrella compacta, C. levicostata, and C. coelastroides (see Supplement Table 1), which could also help to elucidate the phylogenetic relationships among these taxa. We contribute with a new strain isolated from northern Europe, and placed in a culture collection, which can help for further elucidation.

Recently, Wang et al. (2019a) studied the chloroplast genome sequence (cpDNA) of the Chinese strain Coelastrella saipanensis FACHB-2138. This is the first report on the cpDNA structure of the genus Coelastrella. Chloroplast genes often provide stronger phylogenetic signals, however, in the case of Scenedesmaceae, only very few chloroplast genomes are available.

Pigment analysis

Fresh cultures of the strain FGS-001 presented a strong green color due chlorophyll a and b, as main pigments, but after a few weeks under normal conditions, they turned into yellowish-green until reddish-orange after the stationary phase (Fig. 6, Table 2). HPLC–DAD for maximal extinction determination and UPLC-MS for mass detection, were used to determine those carotenoids. We detected the presence of neoxanthin, pheophytin a, astaxanthin, canthaxanthin, lutein, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, α- and β-carotene) under different culture treatments (N, P, sodium acetate, and CO₂).

On one hand, the pigment profile of microalgae have been considered as a relevant taxonomical character in microalgae (Serive et al. 2017), which supported in this case the affiliation of our strain into Coelastrella spp. (Fig. 5) On the other hand, carotenoids are bioactive compounds having characteristic antioxidant, antimicrobial, antiviral, anti-tumoral, anti-inflammatory and anti-allergy effects, which give rise to health benefits (Karpagam et al. 2018).

Habitats where species of Coelastrella grow (with dehydration, temperature stress, salt stress, and high-light exposure) favors the production of pigments and fatty acids. Coelastrella species usually show strong survivability under such extreme photooxidative stresses; and therefore, they are considered good candidate species for large-scale production of natural pigments and biofuels (Wang et al. 2019b).

Fatty acid analysis

Gas chromatography analyses of the FAME-derivatives of the strain FGS-001 showed 17 fatty acids ranging from C10 until C20. The main fatty acids contained 16 and 18 carbon atoms and were identified as linolenic acid, oleic acid and palmitic acid, and palmitoleic acid (Fig. 7, Table 3).

Like other Coelastrella strains, relative fatty acid saturation the percentage of the fatty acids by saturation was high for MUFA and PUFA (Fig. 8). Fatty acid profiles have been considered as another taxonomical character, although we agree with Luo et al. (2016), that the lipid content and fatty acid composition are greatly affected by culturing conditions, growth period, and environmental situation (cf. Hu et al. 2013 and Minyuk et al. 2017).

Thao et al. (2017) studied a strain from Vietnam identified as Coelastrella sp. L3 and registered 17 fatty acids. The main fatty acids were palmitic acid, stearic acid, and oleic acid. In another species isolated from Bulgaria (Coelastrella sp. BGV, Dimitrova et al. (2017) identified as main fatty acids palmitic acid, oleic acid, linoleic acid and α-linolenic acid. Similarly, Abe et al. (2007) analyzed C. striolata var. multisriata isolated from Japan, and found palmitic acid, oleic acid, linoleic acid, and α-linolenic acid. Palmitic acid, α-linolenic acid and linolenic acid were the main fatty acids present in C. rubescens CCALA 475, a strain from Tyrol, Austria (Minyuk et al. 2017). Coelastrella sp. F50 isolated from China presented oleic acid, palmitic acid and linoleic
acid (Hu et al. 2013); and the strain Coelastrella sp. QY01 from China presented palmitic acid, linoleic acid and lino-
lenic acid as main components (Luo et al. 2016). Although
the main fatty acids are shared among the mentioned Coe-
lastrella strains, FGS-001 is the only one with linolenic acid
as the main fatty acid, instead of palmitic acid (ranked third
in our isolate).

We quantified a high percentage of polyunsaturated fatty acids (44–45%) especially linolenic acid (C18:3n3; 30–34%), therefore demonstrating interesting properties for algal biotechnology. As mentioned, the major fatty acids found in the strain were C16–C18, which are commonly found in feedstock suitable for biodiesel production (i.e. oleic acid), in terms of oxidative stability and cold flow properties (Feng et al. 2016).

The genus Coelastrella demonstrates interesting properties for algal biotechnology, but clearly internal relationships among species are not solved yet. We think that a thorough investigation of all those “small and neglected” microalgae groups is necessary, not only for their evolutionary, phylogenetic and ecological implications, but for biotechnology as well. In the case of the present strain FGS-001, it would be interesting to test how it behaves on a larger scale production.

**Conclusion**

We investigated a coccoid green microalgae strain iso-
lated from a terrestrial environment at Ås, Norway, and
used several microscopical and molecular techniques for
its identification. Coelastroideae is a subfamily in Scenedesmaceae still on the way to be clarified its phylogenetic
relationships. The characteristics agree with those of the
genus Coelastrella defined by Chodat in 1922, and the
strain formed a sister group with the recently described C. thermophila var. globula. The fatty acid analyses of the algal biomass showed a high percentage of polyunsaturated fatty acids especially from linolenic acid (30–34%).

While the pigment analysis showed the presence of carot-
enoïds like neoxanthin, pheophytin a, astaxanthin, can-
thaxanthin, lutein, and violaxanthin; the major fatty acids
found in the strain were C16–C18, which are commonly
found in feedstock suitable for biodiesel production.

Therefore, the strain demonstrates interesting properties for algal biotechnology. Coelastrella spp. grow in habitats of high dehydration, temperature stress, salt stress, and
high-light exposure, which apparently favors the produc-
tion of such pigments and fatty acids.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All authors agreed on the publication.

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