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Fatty acids profile of *Mastigocladus laminosus* Cohn ex Kichner isolated from Algerian hot springs as a biofuel feedstock

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ABSTRACT

Cyanobacterial lipids are considered the feedstock of future sustainable biodiesel production, and extremophiles are usually preferred for biotechnological purposes to ease the cultivation problems. However, thermal springs have been scarcely investigated from a biotechnological perspective. Three strains of *Mastigocladus laminosus* Cohn ex Kichner were isolated from hot springs in Algeria, and their fatty acid profiles were studied. The content of saturated fatty acids ranged from 55.91% to 59.37%, while monounsaturated fatty acids and polyunsaturated fatty acids ranged from 38.39% to 43.54% and 0.57%–2.25%, respectively. The main fatty acid was palmitic acid (16:0), with values within the 51.41–53.16% range, followed by oleic acid (18:1n-9) with 24.47–36.60%, and minimal quantities of α-linolenic acid (18:3n-3, below 1%) and long-chain fatty acids (>C18, below 0.5%). The potential biodiesel fuel properties matched the European biodiesel standards EN 14214 (European Norms) and the American standard ASTM D6751 (American Society for Testing and Materials), and were relatively better than other studied cyanobacteria and vegetable oils. The presented data show the interest of *Mastigocladus* and probably other thermophilic strains in different biotechnology fields, especially biofuel production.

1. Introduction

The development of cleaner renewable biofuel technologies is of the greatest interest regarding global warming and the scarcity of energy natural resources (Mondal et al., 2019). Biodiesel has lately received more attention because it is eco-friendly, renewable, non-toxic, and emits fewer gaseous pollutants than conventional fuels (Mostafa and El-Gendy, 2017; Ogunkunle and Ahmed, 2021).

Microalgae are an important alternative source for conventional raw materials thanks to their carbon neutrality, lower hydrocarbon and sulphur emissions, and longer engine lifetime (Mondal et al., 2019; Nagappan et al., 2020). Microalgae with high lipid content, fast growth rate, and extremophile nature may be excellent candidates for biofuel generation (Nagappan et al., 2020).

Cyanobacteria (blue-green algae) are considered a valuable natural source of bioactive compounds and diverse types of metabolites exhibiting anti-cancer, anti-viral, antibacterial, antifungal, and anti-inflammatory activities (Zaki et al., 2021; Righini et al.,

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2022). Moreover, their high lipid production, high cell growth, and adaptability to culture conditions make them ideal lipid sources for biofuel production ((Oliveira et al., 2018; Singh et al., 2019).

Cyanobacteria can generate higher proportion of lipids than plants. Likewise various cyanobacteria can accumulate significant quantities of triacylglyceride (TAG) as a reserve lipid in suitable culturing conditions (ranging from 20 to 50 percent of dry cell weight) (Kumar et al., 2017). Nevertheless, most studies have concentrated on biodiesel production from vegetable oil, mainly palm oil, sunflower oil, sugarcane oil, soybean oil, and other higher plants (Fuad Hossain et al., 2020).

Cyanobacteria live and can adapt to grow in a wide range of extreme environments, including highly saline waters, substrates highly contaminated with metals, deserts, and hot springs (Nandagopal et al., 2021). Hot springs constitute a reservoir of a wide range of microorganisms, most of which are cyanobacteria, synthesizing interesting, and sometimes exclusive, biotechnological products with unique biochemical pathways (Jones and Renaut, 2011; Lukavský et al., 2011). Extremophile organisms have been poorly studied, and therefore, isolating and screening microalgae/cyanobacteria from these under-explored habitats may offer new insights for research and for selecting new strains with high potential for biofuel production, which is not usually the case for other existing biodiesel feedstocks (Mata et al., 2010; Olguin, 2012).

Of all cyanobacteria, the filamentous heterocystous species are very interesting for the production of biomass and chemicals as they can utilise atmospheric nitrogen as the exclusive nitrogen resource, and their filamentous nature offers an advantage for harvesting processes (Anahas and Muralitharan, 2015). Thermophilic filamentous cyanobacteria species modulate their membrane fluidity by inhibiting 16:0 fatty acid (FA) elongations and by increasing monoenoic 16:1 FA production (Los and Mironov, 2015). Cyanobacteria can produce FA with chains longer than 18 carbon atoms, which are present in a small proportion. Vargas et al. (1998) consider that this capacity is not a common characteristic of cyanobacteria.

True-branching filamentous and heterocystous *Mastigocladus laminosus* Cohn ex Kirchner is a good example of an extremophile: it was originally isolated from hot springs and dominates thermal microbial mats in near-neutral pH waters (Kastovsky and Johansen, 2008) with a worldwide distribution (Castenholz, 1976; McGregor and Rasmussen, 2008). *M. laminosus* shows excellent freezing and desiccation tolerance (Castenholz, 1996) and usually synthesises monoenoic FA 14:0, 14:1, 16:0, 16:1, 16:2, 18:0 and 18:1 (Murata et al., 1992; Los and Mironov, 2015).

The FA profile significantly influences the physical and chemical properties of biofuels. Thus, microalgal biodiesel requires certain physical and chemical specifications set by different international standards, such as US ASTM D 6751 Biodiesel Standard in the USA (American Society for Testing and Materials) and EN 14214 Standard in Europe (European Norms) (Prabhu et al., 2019; Uma et al., 2020).

In Algeria, there are more than 240 hot springs with temperatures ranging from 22 °C to 98 °C and located in different areas, mainly in the north-eastern region (Benamara et al., 2017), with different chemical compositions (Foued et al., 2017). The diversity of Algerian thermophilic cyanobacteria has been rarely studied (Amarouche-Yala et al., 2014), and their biotechnological potential remains unexplored. Therefore, this study aims to determine the biotechnological potential for biodiesel production of *Mastigocladus laminosus* isolated from two Algerian hot springs.

2. Material and methods

2.1. Sampling sites and physical-chemical analysis

Two hot springs from north-eastern Algeria with differing ecological features were chosen for this study: the Essalihin spring-Khenchela and the Ouled Ali spring-Guelma (Table 1). Samples were collected from each locality between February and March 2019. Cyanobacteria mat samples were collected in sterile small plastic containers labelled with site name, location code, date, and replicate number. Samples were transported to the laboratory under cold (4 °C) and dark conditions.

Standard physical parameters, including pH, water temperature, dissolved oxygen, and conductivity DO, were measured in the field by a multiparameter probe HORIBA W-23 XD. The water samples for the chemical analysis were collected using 250 mL polyethylene bottles. Mg^{2+}, Na^{+}, K^{+}, Ca^{2+}, HCO^{-}_3, Cl^{-}, SO_4^{2-}, NO^{-}_3, SiO_2, Br, Li^{+} and F^{-} contents were measured with an Atomic Absorption Spectroscopy (AAnalyst 600, Shelton USA) for anions and an Ion Chromatography autosampler (Dionex As40, California USA) for cations, with a precision better than 5%.

2.2. Isolation and enrichment cultures

The isolation of strains was carried out in solid culture media (BG-11 and BBM enriched with soil extract). After 4–8 weeks, the microcolonies grown on a solid medium were streaked on new plates. This process was repeated several times until monoclonal colonies were obtained. All the strains were deposited in the Microalgae edaphic from South-East Spain culture collection (MAESE) located in Murcia University (Spain). Cultures were maintained in a culture chamber (Panasonic MLR-352-PE) at 35 °C, and the light was provided by fluorescent tubes (Panasonic FL 40SS ENW/37 of cool daylight) 16: 8 h light/dark and a light intensity of 75 μM photons m^{-2} s^{-1}. The three strains designated as S4BB*,* S4B11 and S9BB selected to produce enough biomass for lipid extractions were cultivated in 500 mL flasks with liquid medium and aeration and were incubated under the previously indicated conditions for periods of up to 3 weeks. Three replicates were incubated for each strain. Biomass was harvested by sieving via microfiltration using a 40 μm cell strainer (Verma et al., 2010). Biomass production of 9.39, 18.36, and 12.68 g/l was obtained for the strains S4BB*,* S4B11, and S9BB, respectively. The obtained biomass was frozen and maintained at −80 °C until analysis.

Table 1
Physical and chemical characterisation of water from the studied Algerian hot springs.

Hot springs	Ouled Ali	Essalihin
Locality	Guelma	Khenchela
Altitude (masl)	1079	416
Coordinates	35° 26 ′20.12 ″N 7° 05 08.46″ E	36°34′28.63″N 7°22′49.74″E
Temperature (°C)	55.0	57.0
pH	7.0	6.8
Conductivity (mS/m)	2.2	3.7
Dissolved oxygen (mg/l)	16.2	5.3
Ca^{2+} (mg/l)	294.1	199.4
Mg^{2+}(mg/l)	41.0	22.7
Na^{+} (mg/l)	69.0	601.2
K^{+} (mg/l)	7.0	18.0
HCO^{-}_{3} (mg/l)	255.0	295.3
F^{-} (mg/l)	2.2	0.4
Cl^{-} (mg/l)	191.2	692.5
SO_4^{2-} (mg/l)	290.1	254.0
NO^{-}_{3} (mg/l)	0.2	3.9
SiO_2 (mg/l)	50.4	43. 4
Li^{+} (mg/l)	0.1	0.5
Br- (mg/l)	1.2	0.4

2.3. Morphological study and taxonomic identification

The taxonomic identification of field and cultivated cyanobacteria was based on morphological characters observed under a light Olympus (BX50) microscope and photographed with an Olympus U-CMAD-2 camera. The filament morphology and diameter, shape and size of vegetative cells and heterocysts, the position of heterocysts, the appearance of the sheaths, and the presence of hormogonia and akinetes were all recorded. Measurements were performed at different magnifications (40x and 100x). Approximately 50 measurements were recorded for each of the relevant morphological characters. Morphotypes were identified to the species level following the taxonomy of Komárek (2013) and the review of Kastovsky and Johansen (2008).

2.4. Lipid extraction and fatty acids (FA) quantification

Lipids were extracted according to Folch et al. (1957). Briefly, samples were homogenized in 20 mL of chloroform/methanol (2:1 v/v) in a tissue disrupter (IKA ULTRA-TURRAX T 25 digital; IKA-WERKE, Staufen, Germany). Non-lipid impurities were removed by washing with 0.88% KCl. Lipid weight was determined gravimetrically after evaporating the solvent under a stream of nitrogen. FA methyl esters (FAMEs) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie (2003). The total lipid samples were *trans*-methylated overnight in 2 ml of 2% sulphuric acid in methanol (plus 1 ml of toluene to dissolve neutral lipids) at 50 °C. Methyl esters were extracted twice in 5 ml of hexane–diethyl ether (1:1, v/v) after neutralisation with 2 ml of 2% $KHCO_3$, dried under nitrogen and redissolved in 1 ml of iso-hexane. FAMEs were separated and quantified by gas-liquid chromatography in an SP™ 2560 flexible fused silica capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.20 mm SUPELCO) in a Hewlett–Packard 5890 gas chromatograph. The 140 °C oven temperature was initially increased at a rate of 3 °C min-1 to 230 °C, followed by 2 °C min-1, and then to 240 °C to be held for 12 min. The injector and flame ionisation detector were set at 260 °C. Helium was used as the carrier gas at a pressure of 300 kPa. Peaks were identified by comparing their retention times to appropriate FAME standards from Sigma Chemical Company (St. Louis, MO, USA). The data for individual components was expressed as a percentage of total content.

2.5. Predicting biodiesel properties according to the FA profile

Biodiesel properties, including oxidative stability (OS), iodine value (IV), density (ρ), saponification value (SV), degree of unsaturation (DU), cetane number (CN), kinematic viscosity (v), and high heating value (HHV), saturated fatty acid (SFA) levels, monounsaturated fatty acid (MUFA) levels, polyunsaturated fatty acid (PUFA) levels, cold flow properties in the form of cloud point (CP), pour point (PP) filter, plugging point (CFPP), and long-chain saturation factor (LCSF) were estimated by the Biodiesel Analyzer® software, ver. 2.2 (available at “http://www.brteam.ir/biodieselanalyzer”) based on the FA measured in each strain (Talebi et al., 2014). Biofuel properties originating from the cyanobacteria strains were compared to international biodiesel standards to evaluate their quality. Biodiesel fuel qualifications were given by biodiesel standards US ASTM D6751 for the USA and EN 14214 for Europe (ASTM 6751, 2012; EN 14214, 2012).

3. Results and discussion

3.1. Physical and chemical characteristics of the water from the studied hot springs

The temperature of the two thermal springs was 57 °C at the Essalihin spring-Khenchela and 55 °C at the Ouled Ali spring-Guelma. pH values varied from 6.8 (Essalihin spring) to 7.0 (Ouled Ali spring), with electrical conductivities indicating saline water (2.22 and 3.74 mS/m at the Ouled Ali spring and the Essalihin spring, respectively). The studied hot springs can be considered hyperthermal and highly mineralized (Saibi, 2009).

At the Essalihin spring, the main anions were Cl^- and HCO^-_3, while the main cations were Na^+ and Ca^+. The main anions for the Ouled Ali spring were HCO^-_3 and SO_4^{2-}, whereas Ca^+ and Na^+ were the dominant cations (Table 1). The proportion of the major anions and cations was related to the lithological nature of the collection sites, corresponding to various geological complex systems (Chenaker et al., 2018). The Essalhine spring waters correspond to sodium-chloride type from Triassic evaporates (Berkani and Houha, 2017), while the Ouled Ali springs present chloride calcic facies (Chenaker et al., 2018).

3.2. Morphological variation of Mastigocladus laminosus

Three strains of *M. laminosus* were isolated and cultivated: designated as S4BB and S4B11 from the Ouled Ali spring and S9BB from the Essalihine spring. The three strains perfectly match the description of *M. laminosus* (Fig. 1, Table S1) (Komárek, 2013).

Based on a combination of morphology and ecology, the three strains were identified as thermophilic strain *M. laminosus* (Komárek, 2013; Kastovsky and Johansen, 2008). The morphological differences observed among the three isolated strains were probably related to differences in the chemical characteristics of the hot springs and culture media composition (Castenholz, 1976). *M. laminosus* was isolated in this study at temperatures of 55 °C and 57 °C, as referred to in previous records of Miller et al. (2006), Finsinger et al. (2008), Komárek (2013) and Alcamán et al. (2015).

3.3. FA composition

The FA composition in the three strains was equivalent, and no significant variations were detected (Table 2). The SFA content ranged from 55.91% to 59.37%, with MUFA from 38.39% to 43.54%, and the PUFA content ranged from 0.57% to 2.25% (Table 2). The FA profile of the three strains was dominated by 16:0 and 18:1n-9, found at high values ranging from 51.41 to 53.16% and

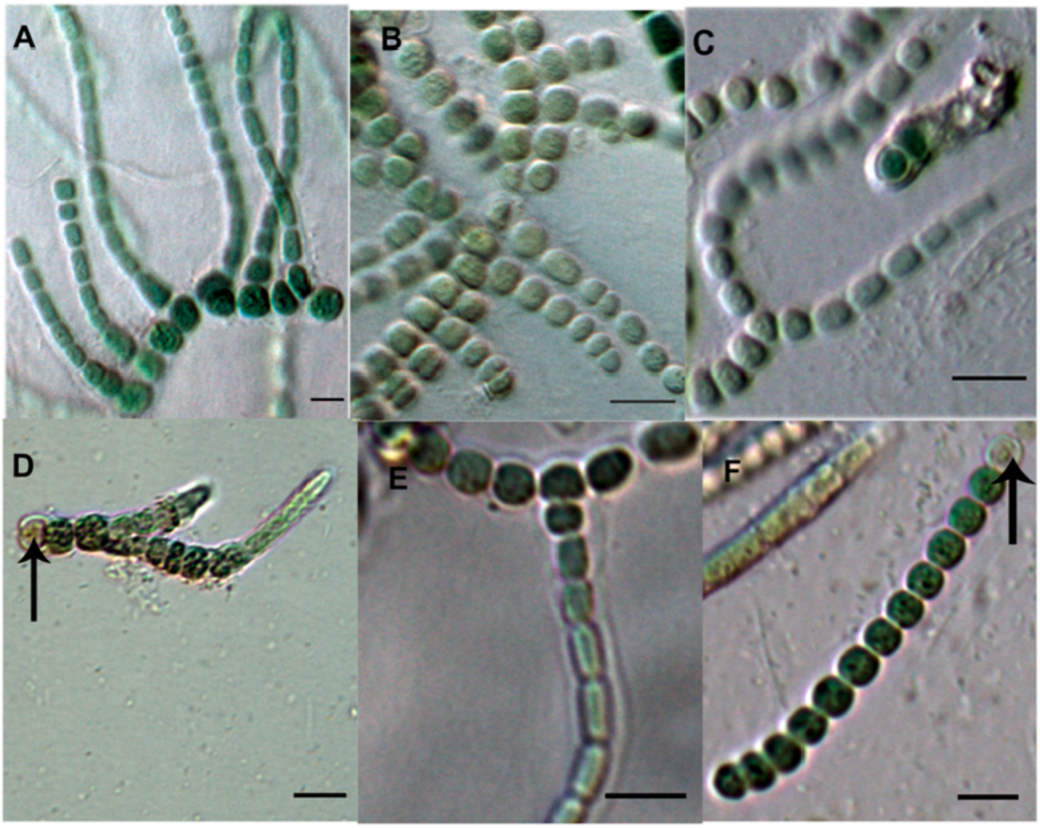

Fig. 1. Morphological variability of *Mastigocladus laminosus* strains. A. Profuse branching of main filament. B. Main filament with two cell rows. C. Y-branching shape. D. Growing branches and a terminal heterocyte. E. T-branching. F. Hormogonium with a terminal heterocyte. Heterocytes are indicated by an arrow. Scale bar = 10 μm.

Table 2
Fatty acid contents of *Mastigocladus laminosus* strains (% wt). Values are the mean ± standard deviations (nd = not detected).

Fatty acids	S4BB	S4B11	S9BB
10:0	0.12 ± 0.07	0.03 ± 0.03	nd
12:0	nd	nd	nd
14:0	1.17 ± 0.09	0.99 ± 0.12	1.38 ± 0.18
15:0	0.18 ± 0.03	0.15 ± 0.03	0.30 ± 0.13
16:0	53.16 ± 0.03	51.41 ± 0.26	52.71 ± 2.46
18:0	3.44 ± 0.64	2.98 ± 0.26	4.40 ± 0.42
20:0	0.13 ± 0.02	0.18 ± 0.04	0.19 ± 0.01
22:0	0.16 ± 0.05	0.17 ± 0.00	0.26 ± 0.01
24:0	0.06 ± 0.01	nd	0.12 ± 0.00
Total SFA	58.42 ± 1.09	55.91 ± 0.16	59.37 ± 1.74
14:1n-5	0.04 ± 0.00	nd	nd
15:1n-5	nd	nd	nd
16:1n-7	7.11 ± 0.37	3.46 ± 0.18	7.25 ± 0.78
18:1n-9	29.46 ± 0.49	36.60 ± 1.33	24.47 ± 1.42
18:1n-7	4.14 ± 0.46	3.44 ± 0.77	6.66 ± 2.00
20:1n-9	0.04 ± 0.00	0.04 ± 0.00	nd
22:1n-9	nd	nd	nd
24:1n-9	nd	nd	nd
Total MUFA	40.78 ± 1.11	43.54 ± 0.38	38.39 ± 1.36
18:2n-6	0.69 ± 0.07	0.32 ± 0.05	1.76 ± 0.28
18:3n-6	0.10 ± 0.01	0.02 ± 0.02	nd
20:2n-6	nd	nd	nd
20:3n-6	nd	nd	0.18 ± 0.04
20:4n-6	0.02 ± 0.01	0.10 ± 0.10	nd
22:2n-6	nd	nd	nd
22:4n-6	nd	nd	nd
Total n-6 PUFA	0.81 ± 0.06	0.44 ± 0.13	1.93 ± 0.32
18:3n-3	0.05 ± 0.01	0.04 ± 0.01	0.21 ± 0.05
18:4n-3	nd	nd	nd
20:3n-3	nd	0.04 ± 0.04	nd
20:5n-3	0.07 ± 0.02	0.06 ± 0.01	0.10 ± 0.00
22:5n-3	nd	nd	nd
22:6n-3	nd	nd	nd
Total n-3 PUFA	0.11 ± 0.02	0.13 ± 0.06	0.31 ± 0.05±
18:1 trans 9	nd	nd	nd
18:2 trans 9–12	nd	nd	nd
Total	nd	nd	nd
Total PUFA	0.92 ± 0.08	0.57 ± 0.19	2.25 ± 0.37
n-3/n-6	0.13 ± 0.01	0.30 ± 0.04	0.16 ± 0.00
ARA/EPA	0.63 ± 0.27	1.61 ± 1.61	0.00 ± 0.00

24.47%–36.60%, respectively, and by moderately high values of 18:0 and 18:1n-7 ranging from 2.98% to 4.40% and from 3.44% to 6.66%, respectively. The values of PUFA like 20:5n-3 (EPA) were relatively low, from 0.06% to 0.10% (Table 2).

In all cases, palmitic (16:0) was the predominant SFA. Oleic (18:1n-9) was the predominant MUFA, and linoleic (18:2n-6) was the most abundant PUFA (0.32–1.76%). Linoleic and arachidonic (ARA, 20:4n-6**)**, with values always lower than 0.10%, were present in all the strains and predominated among n-6, while α-linolenic (ALA, 18:3n-3) and eicosapentaenoic (EPA, 20:5n-3) acids were predominant among n-3 (values were ranging from 0.04 to 0.21% and from 0.06 to 0.10%, respectively) (Table 2). Docosahexaenoic acid (DHA) was not detected in any strain.

Strains of *M. laminosus* showed interesting FA profiles and maybe a promising new potential feedstock for biofuels. The FA compositions in thermophilic cyanobacteria were relatively different from those in common cyanobacteria and other microalgae strains reported in previous studies such as the works of Anahas and Muralitharan (2018), Oliveira et al. (2018), Gayathri et al. (2018) and Lu et al. (2020). The strains isolated in the present study had a high proportion of SFA and MUFA with an average chain length of C14 to C20, which are the major constituents of biodiesel, this makes them extremely valuable for biodiesel production (Sadvakasova et al., 2019; Arif et al., 2019) because higher proportions of MUFA and SFA are favoured in oil profiles (Ortiz-Martínez et al., 2019).

The PUFA detected in all the strains were relatively low. Low PUFA values suggest high-quality fuel produced by these species (Oliveira et al., 2018). Furthermore, the proportion of FA containing four double bonds, or more was well below 0.5% according to biodiesel standards EN 14214; the concentration of FA containing four double bonds must not exceed the 1% limit (Table 2).

In all profiles, the main FA was palmitic acid (16:0), which is typical in most second-generation biodiesel fuels and guarantees excellent biodiesel quality parameters (Oliveira et al., 2018) and was followed by oleic acid (18:1n-9). High oleic acid values suggest better properties for the resulting algal biodiesel (Lee et al., 2014) and increase the biodiesel oxidative stability values for better long-term storage (Mutanda et al., 2011) (Table 2). The α-linolenic acid values (18:3n-3) varied within a narrow range from 0.04% to 0.21%, which is well below the 12% limit set by the EN 14214 standards. Likewise, minimal amounts of long-chain fatty acids (>

C18) were detected and were below 0.10%. It is well known that the lipid content of cyanobacterial cells varies not only by species, but also by growing settings and cell growth phase (Cordeiro et al., 2017). The chemical composition of the culture medium affects the amount of biomass and its lipid composition; thus, the control of the chemical composition of the culture medium is essential to attain increased lipid production (Sharafi et al., 2021), and therefore the culture medium is essential for increased lipid production.

Moreover, the proportion of FA can be modified and enhanced according to biotechnological aims because FA values in algae are influenced by variations in growth conditions (Gonzalez-Silvera et al., 2017). Lipid synthesis can be improved by N_2 starvation, among other stress factors (Kumar et al., 2017), and both low and high temperatures are favoured to achieve elevated lipid levels depending on the strains: the unsaturation rates in FA increase at low temperatures, mainly linoleic and α-linolenic acids (Gonzalez-Silvera et al., 2017; Minhas et al., 2016), while those of total saturated FA rise at high temperatures. The unsaturation level at low temperatures is high, mainly due to a higher dissolved oxygen concentration, allowing oxygen-dependent enzymes, known as omega-3 desaturases, to be active (Minhas et al., 2016). Hence, FA values in cyanobacteria can be changed and enhanced to meet biotechnological goals (Cordeiro et al., 2017; Sharafi et al., 2021).

Furthermore, cyanobacteria biomass is a suitable alternative biomass for energy production since it is renewable and carbon neutral. Hence, elevated biomass yield resulted in high fatty acid harvesting (Fuad Hossain et al., 2020). The three strains of *M. laminosus* showed much higher biomass yields than other *Mastigocladus* strains reported in previous studies, such as *M. laminosus* (CCAP 1447/3) (0.8 g/l) (Smith-Bädorf et al., 2013), *M. laminosus* Sofia (0.53 g/l), *M. laminosus* Carslbad (0.48 g/l) (Rezanka et al., 2012), *Mastigocladus* HS-46 (2.09 g/l) (Prihantini et al., 2021), and in other cyanobacteria species as *Synechococcus* HS-9 (3.323 g/l) (Rahman et al., 2020), *Nostoc carneum* MBDU 709 (0.58 g/l) (Anahas and Muralitharan, 2018), *Synechocystis* PCC 6803 (1.18 g/l), *Synechococcus* PCC 7942 (1.09 g/l), *Nostoc muscorum* (0.76 g/l), *Oscillatoria* sp (0.76 g/l), *Lyngbya* sp (0.64 g/l) and *Phormidium* sp (1.07 g/l) (Patel et al., 2018).

3.4. Biodiesel proprieties

The biodiesel properties of the three studied cyanobacteria strains are found in Table 3, compared to other vegetable and microalgal species, and those for European (EN 14214) and US (ASTM D6751) international standards. The OS, CN, IV and ν values met the requisite international standards recommended by EN 14214 and ASTM D6751 (Table 3) (ASTM 6751, 2012; EN 14214, 2012).

The optimal selection of microalgae species for prospective biodiesel production requires acceptable specifications of FAMEs because biodiesel parameters are influenced mainly by the FA profile (Zaki et al., 2021). The CN values of the studied cyanobacteria biodiesel fell within the authorised range determined by Biodiesel Standards US ASTM D6751 (≥47) and EN 14214 (≥51) (Table 3). CN values ranged from 65.0 to 65, which is the same range as *Desmonostoc muscorum MBDU 105* (CN = 65) but were higher than the values presented by vegetable oils like sunflower and soya bean. *Nostoc calcicola MBDU* 602 had a higher CN value (CN = 201) (Table 3).

The predicted ρ values varied within a narrow range of 834–802 (kg/m^3); the ρ values for the three strains were slightly below the biodiesel standards set by EN 14214 (860–900 kg/m^{-3}) (Table 3). ρ is a crucial fuel specification that influences the engine performance (Arguelles et al., 2018), and SV values ranged from 195.8 to 202.2 mg/g (Table 3). The HHV and LCSF values went from 36.2 to 37.7 (MJ/kg) and 6.5 to 7.4 (wt. %), respectively (Table 3).

The OS values of the studied strains were significantly higher than the limits set by both Biodiesel Standards US ASTM D6751 (≥3 h) and EN 14214 (≥8 h). The OS values were also much higher than all the OS values of the fuel derived from the cyanobacteria and plants cited in Table 3. High CN and OS values are essential advantages for biodiesel storage for long periods (Karatay and Dönmez, 2011). The predicted ν values fell within the viscosity ranges of 1.9–6.0 mm^2/sand 3.5–5.0 mm^2/s given by Biodiesel Standards US ASTM D6751 and EN 14214, respectively (Table 3); this may lead to good combustion, lower emissions, and reduced oil dilution (Knothe, 2008; Arguelles et al., 2018).

The SV and HHV values were consistent with the microalgae values reported in previous studies as the green microalga *Scenedesmus abundans* (Mandotra et al., 2014), *Nostoc spongiaeforme 417 MBDU* 704 and *Nostoc punctiforme MBDU 621* (Gayathri et al., 2018) and with the other plants and cyanobacteria cited in Table 3. The IV values show that the total unsaturation in a fatty acid combination (Arguelles et al., 2018) were lower than the maximum limit set by international standards EN 14214 (≤−120gI$_2$ 100 g^{-1} fat) (Table 3). Neither US ASTM D6751 nor EN 14214 standards specifies values for the HHV, LCSF, SV, and DU criterions (Table 3). High IV value may lead to the occurrence and build-up of glycerides, and grease precipitation in the motor (Arguelles et al., 2018).

CFPP indicates the flow performance of biodiesel at low temperatures (Gayathri et al., 2018). The CFPP values for strains S4BB and S9BB were 5.6 °C and 6.9 °C, respectively, slightly higher than the limit specified by Biodiesel standard EN 14214 (≤5/≤−20 °C) given the high SFA level (Table 3). Whereas, the CFPP value for the S4B11 strain has a value of 4.2 °C (Table 3) meeting the values in EN 14214 biodiesel standards (≤5/≤−20)). High CFPP values are a characteristic of most fuels produced by microalgal species. However, additives or mixing biodiesel with petrodiesel can be used to lower CFPP values (Knothe, 2008).

Overall, the studied thermophilic *Mastigocladus* strains had better biodiesel proprieties, or they fell within the same range of those reported for other plants or cyanobacteria feedstocks. The obtained values met the required fuel properties of international biodiesel standards (Table 3), along with SFA dominance, showing that the studied cyanobacteria are favourable for producing high-quality biodiesel (Oliveira et al., 2018).

Efforts for the characterisation of algae from unique environments should continue for both biodiversity conservation and biotechnology goals (Hu et al., 2008), isolating microalgae with high productivity and interesting lipid composition from specific and extreme habitats and optimising their growth conditions to generate high biomasses for biodiesel production (Lee et al., 2014; Arif et al., 2019) or other potentially interesting compounds.

Table 3
Comparison of predicted biodiesel properties from FA profiles of the three cyanobacterial strains of this study compared to others selected fuel producer organisms.

Cyanobacterial strains	*Biodiesel standard ASTM* D6751	*Biodiesel standard EN 14214*	*Mastigocladus laminosus* S4BB This study	*Mastigocladus laminosus* S4B11 This study	*Mastigocladus laminosus* S9BB This study	*Nostoc calcicola MBDU 602* (Anahas and Muralitharan, 2018)	*Desmonostoc muscorum MBDU 105* (Anahas and Muralitharan, 2018)	*Myxosarcina* sp. (Jawaharraja et al., 2015)	Algae (Yaşar, 2020)	Soybean (Yaşar, 2020; Demirbaş, 2003)	Sunflower (Yaşar, 2020; Demirbaş, 2003)
Fatty Acid Composition (% wt).	/	/	SFA 58.07 MUFA 36.61 PUFA 0.79	SFA 55.47 MUFA 40.10 PUFA 0.34	SFA 58.79 MUFA 31.72 PUFA 1.76	SFA 61.78 MUFA 6.46 PUFA 5.56	SFA 66.30 MUFA 8.25 PUFA 13.62	SFA 39 ± 5.3 MUFA 61 ± 5 PUFA nd	SFA 18.33 MUFA 56.21 PUFA 25.44	SFA 16.34 MUFA 24.91 PUFA 58.66	SFA 10.14 MUFA 36.37 PUFA 53.33
IV (gI_2/100 g fat)	/	≤120	35.00	37.00	32.43	23.73	40.36	56.00	/	69.82	132.32
CN	≥47	≥51	65.00	65.02	66.87	201.50	65.39	60.00	59.00	38.10	50.00
OS (hrs)	≥3	≥8	151.00	349.44	69.59	7.09	6.40	nd	2.30	1.50	0.90
DU (wt. %)	/	/	38.10	40.78	35.24	17.59	35.50	61.00	107.09	142.25	143.02
SV (mg/g)	/	/	202.20	201.76	195.84	68.04	193.73	210.00	/	220.78	34.40
ν (mm^2/s)	1.9–6.0	3.5–5.0	3.60	3.69	3.47	2.15	3.96	−1.50	4.55	4.37	4.30
ρ (kg/m^3)	/	860–900	830.00	834.00	802.00	880.00	870.00	1100.00	881.00	882.00	882.00
LCSF (wt. %)	/	/	7.00	6.59	7.47	58.36	22.71	6.00	5.36	3.73	3.23
CFPP (°C)	/	≤5/≤−20	5.60	4.21	6.99	166.89	54.89	2.00	−14.00	−6.00	−4.00
HHV (MJ/kg)	Report	Report	37.50	37.75	36.27	40.81	40.88	35.00	–	39.60	39.60

4. Conclusion

The strains of *M. laminosus* isolated from the Algerian hot springs showed an interesting FA profile under laboratory conditions that may be suitable for mass cultivation and could represent a source of essential FA of commercial interest. They exhibit a suitable FA profile with high proportion of SFA and MUFA with values ranging from 55.91% to 59.37%, and 38.39%–43.54%, respectively. The main fatty acid was palmitic acid (16:0), with values within the 51.41–53.16% range and with an average chain length of C14 to C20, representing an excellent raw material for biodiesel production. The calculated biodiesel fuel properties based on the fatty acid composition mainly the iodine value (IV, 35.00 gI_2/100 g fat), saponification value (SV, 202.20 mg/g), cetane number (CN, 65.00) and unsaturation degree (DU, 38.10 wt %) were in accordance with the international standards ASTM D6751 (USA) and EN 14214. Hot springs in Algeria and elsewhere should be more deeply studied to better understand their biodiversity and their potential biotechnological uses.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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