

# Screening and Characterization of Oleaginous Microalgal Species from Northern Xinjiang

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A total of 646 strains, including green algae and diatoms, were isolated from 220 samples to screen microalgae with high lipid productivity (LP). The samples were obtained from nine habitats in Northern Xinjiang, China in June 2013. This study initially identified eight lipid-rich strains, namely, *Desmodesmus intermedius* XJ-498, *D. intermedius* XJ-145, *D. intermedius* XJ-99, *Monoraphidium pusillum* XJ-489, *M. dybowskii* XJ-435, *M. dybowskii* XJ-151, *Mychonastes homosphaera* XJ-488, and *Podohedriella falcata* XJ-176, based on 18S rDNA sequencing. The strains were cultured in a photobioreactor for the same period. Results showed that the specific growth rate ( $\text{day}^{-1}$ ) of *M. pusillum* XJ-489 was the highest ( $1.14 \pm 0.06$ ), and the biomass concentration (g/l) of *D. intermedius* XJ-99 was the highest ( $2.84 \pm 0.3$ ). Furthermore, the lipid content (%) of *M. dybowskii* XJ-151 was the highest ( $33.5 \pm 4.38$ ), and the lipid productivity ( $\text{mg l}^{-1} \text{day}^{-1}$ ) of *My. homosphaera* XJ-488 was the highest ( $86.41 \pm 9.04$ ). C16 to C18 accounted for 86% to 98% of the total lipid, and the biodiesel qualities of the selected algae corresponded to international standards. This study suggests that *My. homosphaera* XJ-488, *D. intermedius* XJ-99, and *M. dybowskii* XJ-151 are the most potential strains for biodiesel production among all the isolated strains.

**Keywords:** Microalgae, screening, lipid productivity, fatty acid profiles, biodiesel quality

## Introduction

With the increasing energy crisis and deteriorating environment induced by using fossil energy, exploring renewable, environmentally friendly, and economically viable alternative fuels has become an important strategic direction of world energy [20]. Third-generation biodiesel feedstocks derived from microalgae have emerged as one of the most promising alternative sources [2]. With the deep understanding of microalgae, the problem of microalgae germplasm resources is becoming increasingly prominent; algal species almost become the main factors that restrict the development of the bioenergy industry [7, 23]. Therefore, screening for indigenous oleaginous microalgae with a rapid growth rate and high lipid productivity (LP) is the key to support this technique [24].

Good strains are not only known to meet the requirement of high lipid content (LC), high biomass concentration, and

ease in harvesting, but also known to have strong environmental tolerance and good biodiesel quality [10, 11, 22].

Xinjiang Uygur Autonomous Region is the largest province of China. This region lies in the northwest part of China, and is the center of the Eurasian continent. Given its location in the inland and mountain barrier surrounding the area, the region has to a typical temperate continental arid climate. Considering the vast territory, complicated terrain, and different climate along with different heights and longitudes, Northern Xinjiang was chosen as the study region. Different types of water (lakes, rivers, ditches, ponds, and reservoirs) and soil (deserts, meadows, farmlands, and shallows) samples were collected to obtain sufficient representative samples and more excellent strains. After separation and purification of microalgae, the biomass concentration and lipid accumulation of the purified strains were immediately examined. Strains with LP higher than  $40 \text{ mg l}^{-1} \text{day}^{-1}$  were identified by 18S rDNA sequence

analysis. This study also analyzed the fatty acid (FA) profiles and biodiesel quality of the selected algae.

## Materials and Methods

### Survey Region and Sampling

Northern Xinjiang, including the Junggar Basin, lies in the north of the Tianshan Mountain and south of the Altai Mountain, with a total area of more than 60 km<sup>2</sup>. Snowmelt from the mountain forms several continent rivers, flowing into depressions of the basin, except the Eltrix River, which flows into the Arctic Ocean. The altitude of the territory is variable, with an average altitude of less than 500 m, and Aydingkol Lake has the lowest altitude (161 m below sea level). The central part of the basin is the Gurbantunggut Desert, which is the second largest desert in China. The majority of this area is fixed or semi-fixed sand dunes. The western basin is the Ili area, with more mountains than plains and cold temperate semi-arid continental climate.

In this study, a total of 220 algae samples (185 water samples and 35 soil samples) were collected from nine habitats. The samples in the same habitat were collected at a distance of at least 15 km. The location, pH, temperature, soil property, and vegetation condition of the sampling site were also recorded.

### Isolation and Purification of Microalgae

BG11 medium was used to incubate freshwater microalgae and most soil microalgae. F/2 medium was used to incubate saline microalgae. Agar plates and micropipettes were employed to isolate algae strains. Each purified strain was initially cultured in a test tube (30 ml) at 25 ± 1°C under continuous illumination using white fluorescent light at an intensity of 70 μmol s<sup>-1</sup> m<sup>-2</sup> for 1 to 3 weeks. The strains were then incubated into a 100 ml flask with sterile air at 25 ± 1°C under an intensity of 80 μmol s<sup>-1</sup> m<sup>-2</sup> for 10 days.

### Identification by 18S rDNA Sequence

Microalgae strains were initially identified with a microscope and then confirmed by using 18S rDNA sequence analysis. Algae were harvested in the exponential growth phase by centrifugation (4,000 ×g, 10 min), and the cells were completely ground by liquid nitrogen. Total DNA was initially extracted using a Plant Genomic DNA kit, analyzed by electrophoresis in 1% agarose gel to confirm the presence and concentration of products, and then used for PCR amplification (Bio-Rad Thermal Cycler, USA). Amplification reactions used the following universal eukaryotic primers: forward, 5'-TTCGGCTAGGGATAGGCTTG-3'; and reverse, 5'-TTTGAT TTCTCATAAGGTGC-3'. The PCR products were analyzed by electrophoresis in 1% agarose gel and sent to the sequencing facility (WuHan Tingke Biotech Co., Ltd., China) for the corresponding analysis. 18S rDNA sequences of the isolates were searched against GenBank using BLAST for homologous analysis, according to similarities by the Clustal W program. Phylogenetic trees were constructed using the neighbor-joining method from the Molecular

Evolutionary Genetics Analysis ver. 5 software after 1,000 bootstrap replicates.

### Microalgal Cultivation

The capacity of growth and lipid production of strains was investigated. Eight microalgae strains were selected and cultivated in a column bioreactor (30 mm × 600 mm), containing 250 ml of BG11 medium with aeration by sterile air, under a 14 h:10 h light:dark photoperiod of white fluorescent light (100 ± 2 μmol m<sup>-2</sup> s<sup>-1</sup>) at 25 ± 1°C for 10 days. The algal density was determined by measuring the OD<sub>680</sub> (the optical density of algal at 680 nm) with TU-1900 UV spectroscopy.

The cells were harvested by centrifugation (8,000 ×g, 5 min) and lyophilized using a vacuum freeze dryer (Alpha 1-2 LD plus; Christ). Each experiment was conducted in triplicate.

### Lipid Analysis

The total lipid was extracted from approximately 50 to 100 mg of dried algae (W<sub>0</sub>) using a Soxhlet apparatus, with chloroform-methanol (1:2 (v/v)) as the solvent at 90°C for 4 to 6 h [5, 15]. The total lipid was transferred into a pre-weighed grass dish (V<sub>1</sub>), dried to a constant weight in an oven at 65°C, and weighed (V<sub>2</sub>).

The lipid content (LC, %) and the lipid productivity (LP, mg l<sup>-1</sup> day<sup>-1</sup>) were determined according to Eqs. (1) to (2):

$$LC = 100 \times (V_2 - V_1) / W_0 \quad (1)$$

$$LP = LC \times BP \quad (2)$$

where BP represents the biomass productivity (mg l<sup>-1</sup> day<sup>-1</sup>).

### Analysis of Fatty Acid Profiles

The methanolysis of FAs was performed with 1 M H<sub>2</sub>SO<sub>4</sub>-CH<sub>3</sub>OH at 100°C for 1 h using the modified Davila method [6]. FA components of extracted lipid were analyzed by gas chromatography mass spectrometry (Ultra trace, Thermo Scientific ITQ 700, USA) equipped with a fused silica capillary column (60 mm × 0.25 mm × 0.25 μm; Agilent Technologies, USA) and a flame ionization detector (FID). The initial temperature was maintained at 50°C for 1 min and then increased to 170°C at 40°C/min. The oven temperature was raised from 170°C to 210°C at 18°C/min after a 1 min hold. The injector temperature was 270°C, and nitrogen was used as a carrier gas with a flow rate of 2.0 ml/min. The detector temperature was 280°C, and air and hydrogen flows were set at 350 and 35 ml/min, respectively. All the parameters of FA methyl ester (FAME) were derived from the calibration curves generated from the FAME standard mix (Supelco 37 Component FAME Mix; Sigma-Aldrich, USA).

### Estimation of Biodiesel Properties

In recent years, studies have focused on estimating the quality parameters of biodiesel according to the FA components [13, 19], and the detailed calculation [21] is as follows:

$$ADU = \sum M \times Y_i \quad (3)$$

where ADU is the average degree of unsaturation of microalgal oil; M is the number of carbon-carbon double bonds in each FA component; and Yi is the mass fraction of each FA component.

The relationships between ADU and other biodiesel properties, namely, kinematic viscosity (KV), specific gravity (SG), cloud point (CP), cetane number (CN), iodine value (IV), and higher heating value (HHV) as well as the cold filter plug point (CFPP) and long-chain saturated factor (LCSF) were all determined by empirical equations from FA composition as described previously [9, 19].

### Statistical Analysis

The data were analyzed by one-way ANOVA and cluster analysis using SPSS statistical software (ver. 19.0).  $P < 0.05$  denotes a statistically significant difference. The values were expressed as the mean  $\pm$  standard deviation.

## Results and Discussion

### Isolation and Identification

Separation of algal strains is a necessary prerequisite for screening [8]. A total of 646 algal strains, including green algae and diatoms, were initially isolated from 220 samples in Northern Xinjiang and then cultivated in 100 ml flasks. Eight cultured microalgae with LP higher than  $40 \text{ mg l}^{-1} \text{ day}^{-1}$  were selected for further study (Table 1).

After the morphological analysis, phylogenetic trees were created according to the BLAST analysis of corresponding sequences (Fig. 1). The eight microalgae strains were respectively named as *Desmodesmus intermedius* XJ-498, *D. intermedius* XJ-145, *D. intermedius* XJ-99, *Monoraphidium pusillum* XJ-489, *M. dybowskii* XJ-435, *M. dybowskii* XJ-151, *Mychonastes homosphaera* XJ-488, and *Podohedriella falcata* XJ-176.

### Growth Characteristics

The eight strains were cultured in a column photobioreactor

for 10 days. Comparison between the specific growth rates (Table 2) showed that two strains from saline environment were lower than the freshwater strains. The specific growth rate ( $\text{day}^{-1}$ ) of *M. pusillum* XJ-489 was the highest ( $1.14 \pm 0.06$ ), followed by *P. falcata* XJ-176 ( $1.08 \pm 0.16$ ), which was higher in previous reports [11, 21]. The specific growth rate of *M. dybowskii* XJ-435 was the lowest and significantly lower than the other strains ( $p < 0.05$ ). The biological growth potential of *M. pusillum* XJ-489 and *P. falcata* XJ-176 was great, whereas the growth of the two algae strains from saline environment was unsatisfactory. This result suggests that the medium conditions were inappropriate and need further study.

*D. intermedius* XJ-99 showed the highest biomass concentration ( $2.84 \pm 0.3 \text{ g/l}$ ) (Table 2), which was higher than the reported *Desmodesmus* sp. WC08 [23] (Table 2); except for *M. dybowskii* XJ-435, the biomass concentrations of other strains were over  $2 \text{ g/l}$ . This result indicates that the algae from the common fresh water grew faster than those from the stress environment when cultured under the present conditions.

### Lipid Content and Lipid Productivity

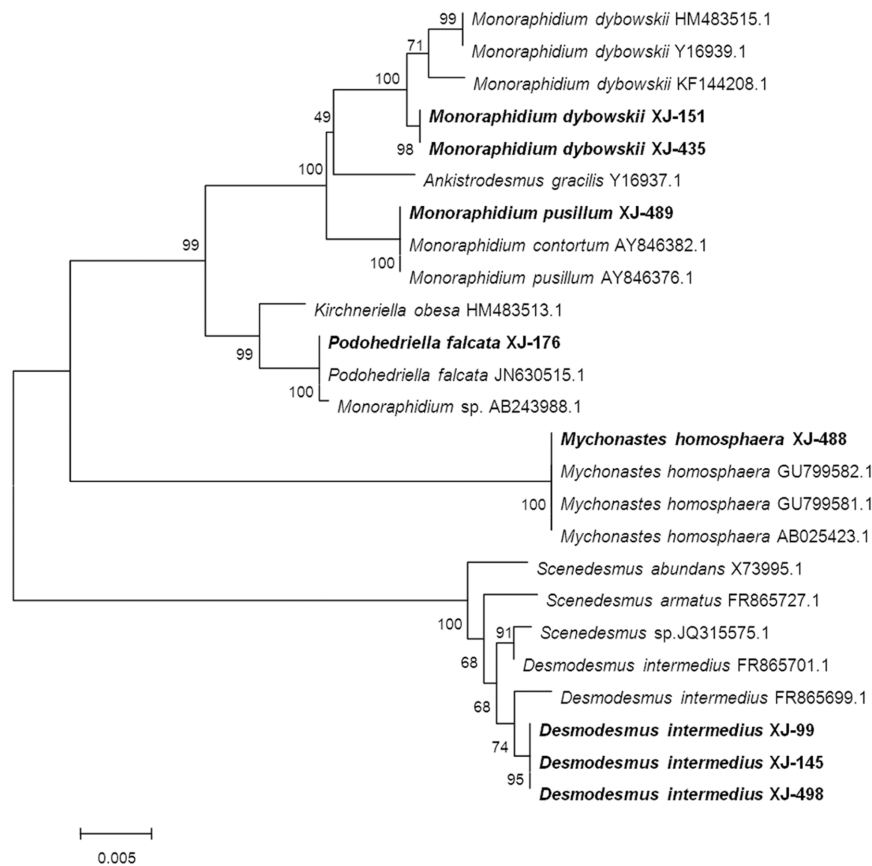
After being cultured, the total lipid contents of the strains ranged from 22% to 33% (Table 2). The LC (%) of *M. dybowskii* XJ-151 was the highest ( $33.5 \pm 4.38$ ), followed by *M. dybowskii* XJ-435 ( $33.2 \pm 0.57$ ), which was higher than the literature data for *M. dybowskii* [3] (Table 2).

Comparison of the lipid productivity ( $\text{mg l}^{-1} \text{ day}^{-1}$ ) of the eight tested strains (Table 2) showed that *D. intermedius* XJ-99 was the highest ( $86.41 \pm 9.04$ ), followed by *My. homosphaera* XJ-488 ( $85.58 \pm 6.24$ ). The LP ( $\text{mg l}^{-1} \text{ day}^{-1}$ ) of *D. intermedius* XJ-498 ( $52.56 \pm 13.34$ ) and *D. intermedius* XJ-145 ( $52.35 \pm 4.88$ ) was the lowest. Compared with previous reports, the strains were higher than *Scenedesmus* sp. [1, 19], but lower than *Desmodesmus* sp. WC08 [23] (Table 2). Consistent with previous reports, several strains with high LC presented low

**Table 1.** Habitat information of eight microalgal strains.

Strains	Sampling location	Type of habitat	Type of water	Altitude (m)	Temperature (°C)	pH
<i>Desmodesmus intermedius</i> XJ-498	44.30N, 86.36E	Reservoir	Salt water	435	16	7.2
<i>Desmodesmus intermedius</i> XJ-145	43.84N, 80.67E	River	Fresh water	530	16	7
<i>Desmodesmus intermedius</i> XJ-99	44.63N, 84.61E	Reservoir	Fresh water	328	16	6.8
<i>Monoraphidium dybowskii</i> XJ-435	42.69N, 89.25E	Lake	Salt water	-161	20	7
<i>Monoraphidium dybowskii</i> XJ-151	43.84N, 80.67E	River	Fresh water	530	16	7
<i>Monoraphidium pusillum</i> XJ-489	47.75N, 87.51E	River	Fresh water	516	17	7
<i>Mychonastes homosphaera</i> XJ-488	47.75N, 87.51E	River	Fresh water	516	17	7
<i>Podohedriella falcata</i> XJ-176	44.30N, 86.36E	Reservoir	Fresh water	436	15	6.5

"E" and "N" represent east longitude and north latitude, respectively.



**Fig. 1.** Phylogenetic tree of the 18S rDNA of eight microalgal strains.

The topology of this tree was constructed using the neighbor-joining method after 1,000 rounds of bootstrap resampling. The tested algae are indicated in bold font.

biomass concentration. Although no necessary connection was found between biomass concentration and LC [12, 19], this phenomenon still affected the application prospect of these strains. In terms of LP, *My. homosphaera* XJ-488 and *D. intermedius* XJ-99 were the more promising strains for lipid production. In terms of resisting to pollution from the outdoor culture, *D. intermedius* XJ-498 and *M. dybowskii* XJ-435 may have potential, but these saline strains still need further study.

#### Fatty Acid Profiles and Biodiesel Properties

Screening of better oleaginous microalgae should not only consider the LP, but also the composition and quality attribute of FAs for biodiesel production [14]. The main components of biodiesel are saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) [4]. The major FA compositions of green algae are C16 to C18, which are suitable for biodiesel production [16]. The results (Table 3) showed that the overall FA compositions were similar,

where the main components of FA were C16 and C18. The contents of C16 to C18 were 86% to 98%, whereas the long-chain FAs above C20 exhibited low quantities. By comparison, SFAs of *M. dybowskii* XJ-435 and *D. intermedius* XJ-99 showed the highest proportion (41.09% and 41.94%, respectively). Polyunsaturated fatty acid (PUFA) contents among them were different; the highest was 37.95% (*D. intermedius* XJ-498) and the lowest was only 9.81% (*D. intermedius* XJ-99). *D. intermedius* XJ-498 exhibited the lowest C18:1 content (18.15%), but had the highest C18:3 content (18.18%). However, C18 FA contents composed of PUFAs in algal oils were much less than in vegetable oils [18]. The aforementioned results showed that the FA compositions of the eight strains were suitable for biodiesel production.

Based on FA compositions, this study also estimated several important qualities of the strains as biodiesel (Table 4). According to the present knowledge, with increasing values of ADU and CN, its oxidation stability is lower; and the high IV will be easily oxidized to form sediments, affecting

**Table 2.** Comparison of biomass and lipid productivity of some microalgae in different cultivated conditions.

Strains	$\mu$ (day <sup>-1</sup> )	BC (g/l)	BP (mg l <sup>-1</sup> day <sup>-1</sup> )	LC (%)	LP (mg l <sup>-1</sup> day <sup>-1</sup> )	Culture	Bioreactor	Source
<i>D. intermedius</i> XJ-498	0.78 ± 0.05	2.19 ± 0.5	218.77 ± 49.84	23.95 ± 0.64	52.56 ± 13.34	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>D. intermedius</i> XJ-145	0.99 ± 0.08	2.33 ± 0.07	233.02 ± 7.43	22.45 ± 1.38	52.35 ± 4.88	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>D. intermedius</i> XJ-99	0.87 ± 0.09	2.84 ± 0.3	283.16 ± 30.58	30.28 ± 1.06	85.58 ± 6.24	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>M. dybowskii</i> XJ-435	0.49 ± 0.08	1.85 ± 0.15	184.94 ± 15.37	33.2 ± 0.57	61.44 ± 6.15	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>M. dybowskii</i> XJ-151	0.84 ± 0.07	2.51 ± 0.21	251 ± 21.21	33.5 ± 4.38	84.59 ± 18.26	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>M. pusillum</i> XJ-489	1.14 ± 0.06	2.06 ± 0.06	210.29 ± 5.64	31.55 ± 0.13	66.34 ± 1.51	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>My. homosphaera</i> XJ-488	0.85 ± 0.1	2.82 ± 0.21	281.54 ± 19.95	30.66 ± 1.04	86.41 ± 9.04	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>P. falcata</i> XJ-176	1.08 ± 0.16	2.3 ± 0.84	230.21 ± 84.17	29.92 ± 2.61	69.97 ± 31.2	Air	Transparent glasstube (600 mm × 30 mm)	This study
<i>D. subspicatus</i> WC01	-	1.47 ± 0.1	147.2 ± 9.96	31.1 ± 1.2	47.56 ± 2.4	Air	Transparent glasstube (600 mm × 30 mm)	[23]
<i>Desmodesmus</i> sp. WC08	-	2.32 ± 0.59	370 ± 22.99	31.3 ± 1	115.73 ± 5.89	Air	Transparent glasstube (600 mm × 30 mm)	[23]
<i>D. brasiliensis</i>	0.28	-	130	17.99 ± 0.42	23.39 ± 0.63	2% CO <sub>2</sub>	Erlenmeyer flasks (1 L)	[19]
<i>Scenedesmus obliquus</i>	0.21	-	160	16.73 ± 1.37	26.77 ± 2.53	2% CO <sub>2</sub>	Erlenmeyer flasks (1 L)	[19]
<i>Ankistrodesmus falcatus</i>	0.57	-	340	16.49 ± 0.44	56.07 ± 1.75	2% CO <sub>2</sub>	Erlenmeyer flasks (1 L)	[19]
<i>Ankistrodesmus fusiformis</i>	0.39	-	240	20.66 ± 2.07	49.58 ± 5.74	2% CO <sub>2</sub>	Erlenmeyer flasks (1 L)	[19]
<i>Scenedesmus obliquus</i> YSL02	-	1.84 ± 0.3	-	29	-	Normal	Erlenmeyer flasks (250 ml)	[1]
<i>Scenedesmus obliquus</i> YSL05	-	1.71 ± 0.53	-	28	-	Normal	Erlenmeyer flasks (250 ml)	[1]
<i>Scenedesmus obliquus</i>	0.16	0.21	-	-	6.57	Normal	Erlenmeyer flasks (500 ml)	[21]
<i>M. dybowskii</i> (SAG 202-7e)	-	-	306	19	-	3% CO <sub>2</sub>	Glass bottles (3 L)	[3]
<i>M. contortum</i> (SAG 47.80)	-	-	307	22.2	-	3% CO <sub>2</sub>	Glass bottles (3 L)	[3]

$\mu$ , the special growth rate; BC, biomass concentration; BP, biomass productivity; LC, lipid content; LP, lipid productivity.

its lubrication [19]. Thus, these properties are particularly important. In terms of the two common quality standards for biodiesel, namely, ASTM D6751 in the US and EN 14214 in Europe, all eight strains satisfied the standards. Given that their CFPP was below  $-15^{\circ}\text{C}$ , the strains have better low-temperature fluidity [17]. Furthermore, the SFAs and

MUFAs of these strains accounted for a high proportion, resulting in better diesel oxidation stability.

This research combined LP and biodiesel properties by cluster analysis to comprehensively evaluate the potential of the eight strains for biodiesel production [9, 19]. The results showed that *My. homosphaera* XJ-488, *D. intermedius*

**Table 3.** Fatty acid profiles of eight microalgal strains (% of total FAMES).

FA profiles	XJ-498	XJ-145	XJ-99	XJ-435	XJ-151	XJ-489	XJ-488	XJ-176
14:0	2.71	1.81	1.73	1.15	0.72	0.59	0.39	1.08
14:1	4.21	1.41	1.06	1.62	1.29	0.86	0.59	1.47
16:0	26.11	26.36	37.73	32.59	30.53	27.39	26.49	25.48
16:1	3.40	8.20	2.47	5.69	4.92	4.39	8.50	1.02
18:0	3.67	4.48	1.38	4.25	3.32	5.31	2.01	6.24
18:1	18.15	34.55	44.72	27.27	43.02	33.11	27.19	34.45
18:2	15.19	7.32	1.47	11.34	9.37	21.39	22.93	13.21
18:3	18.18	9.63	5.96	11.47	4.17	4.51	10.50	11.39
18:4	1.25	1.34	0.81	0.29	0.71	0.55	0.32	1.14
20:0	2.89	1.65	0.50	1.84	0.76	0.62	0.48	0.69
20:4	1.05	1.22	0.90	1.08	0.52	0.35	0.19	1.19
20:5	2.28	0.48	0.66	0.15	0.41	0.78	0.40	1.31
22:0	0.91	1.54	0.61	1.25	0.27	0.16	0.00	1.31
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
SFA	36.29	35.85	41.94	41.09	35.59	34.06	29.38	34.81
MUFA	25.76	44.16	48.25	34.57	49.23	38.36	36.28	36.95
PUFA	37.95	19.98	9.81	24.33	15.18	27.58	34.34	28.24
C16-C18	85.96	91.88	94.55	92.90	96.02	96.64	97.94	92.94

XJ-498, *Desmodesmus intermedius* XJ-498; XJ-145, *Desmodesmus intermedius* XJ-145; XJ-99, *Desmodesmus intermedius* XJ-99; XJ-435, *Monoraphidium dybowskii* XJ-435; XJ-151, *Monoraphidium dybowskii* XJ-151; XJ-489, *Monoraphidium pusillum* XJ-489; XJ-488, *Mychonastes homosphaera* XJ-488; XJ-176, *Podohedriella falcata* XJ-176.

**Table 4.** Comparison of biodiesel properties between the selected strains, and ASTM biodiesel and EN biodiesel standards.

Property	XJ-498	XJ-145	XJ-99	XJ-435	XJ-151	XJ-489	XJ-488	XJ-176	ASTM D6751-08	EN 14214
ADU	1.31	1	0.79	0.98	0.87	1.02	1.18	1.13	-	-
KV 40°C (mm <sup>2</sup> /s)	4.38	4.57	4.71	4.59	4.65	4.56	4.46	4.49	1.9-6.0	3.5-5.0
SG (kg/l)	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.85-0.90	-
CP (°C)	2.46	6.6	9.41	6.92	8.32	6.35	4.28	4.85	-	-
CN	54.12	56.19	57.59	56.35	57.05	56.06	55.03	55.31	Min47	Min51
IV (g I <sub>2</sub> /100 g)	110.34	87.31	71.63	85.52	77.74	88.69	100.23	97.06	-	Max120
HHV (MJ/kg)	40.84	40.3	39.93	40.26	40.07	40.33	40.61	40.53	-	-
CFPP (°C)	-15.26	-15.29	-15.4	-15.34	-15.35	-15.3	-15.34	-15.19	-	-

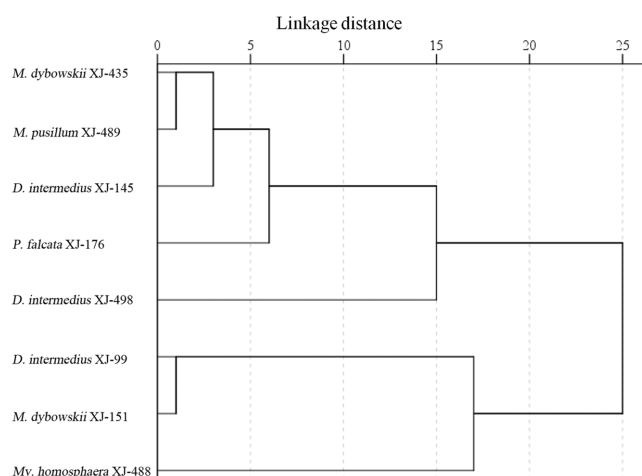
ADU, average degree of unsaturation; KV, kinematic viscosity; SG, specific gravity; CP, cloud point; CN, cetane number; IV, iodine value; HHV, higher heating value; CFPP, the cold filter plug point; LCSF, long-chain saturated factor.

XJ-99, and *M. dybowskii* XJ-151 could be classified into one category (Fig. 2), because of their high LPs and similar biodiesel qualities. Therefore, these strains are the most promising for further study.

In conclusion, among the 646 strains (543 strains from water samples and 103 strains from soil samples), eight strains with high LP were selected from the aquatic environment. After being cultured in a photobioreactor, *M. pusillum* XJ-489 reached the highest specific growth rate ( $1.14 \pm 0.06 \text{ day}^{-1}$ ), *D. intermedius* XJ-99 reached the highest biomass concentration

( $2.84 \pm 0.3 \text{ g/l}$ ), *M. dybowskii* XJ-151 reached the highest LC ( $33.5 \pm 4.38\%$ ), and *My. homosphaera* XJ-488 reached the highest LP ( $86.41 \pm 9.04 \text{ mg l}^{-1} \text{ day}^{-1}$ ). The C16 to C18 contents of the eight strains were over 86%, and the biodiesel qualities of the selected algae corresponded to international standards. Considering their LP and biodiesel quality, this study suggests that *My. homosphaera* XJ-488, *D. intermedius* XJ-99, and *M. dybowskii* XJ-151 are the most potential strains for biodiesel production.

Prior to large-scale application, finding more effective ways



**Fig. 2.** Cluster analysis comparing the variation of microalgae lipid productivity and biodiesel properties.

of cultivation and evaluating the ability of the strains to resist pollution are also necessary. *D. intermedius* XJ-498 from a saline environment and *M. dybowskii* XJ-435 with better growth potential need to be further investigated as promising strains.

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