Polyphasic characterization of the thermotolerant cyanobacterium Desertifilum sp. strain IPPAS B-1220

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One sentence summary: Polyphasic characterization of a new thermophilic cyanobacterial strain of Desertifilum, IPPAS B-1220, revealed an unusual fatty acid composition and the presence of six genes for the acyl-lipid desaturases.

Editor: Aharon Oren

ABSTRACT

A cyanobacterial strain from Lake Shar-Nuur, a freshwater lake in Mongolia, was isolated and characterized by a polyphasic approach. According to the 16S ribosomal RNA gene sequence, this strain (IPPAS B-1220) belongs to a newly described genus Desertifilum. In general, strains of Desertifilum maintain their genetic stability, as seen from the analysis of the 16S rRNA gene and 16S–23S rRNA internal transcribed spacer sequences from strains collected at distant locations. The newly discovered strain is characterized by an unusual fatty acid composition (16:1Δ7 and 16:2Δ7,10). Analysis of its draft genomic sequence reveals the presence of six genes for the acyl-lipid desaturases: two Δ9-desaturases, desC1 and desC2; two Δ12-desaturases, desA1 and desA2; one desaturase of unknown specificity, desX; and one gene for the bacillary-type desaturase, desG, which supposedly encodes an ω9-desaturase. A scheme for a fatty acid desaturation pathway that describes the biosynthesis of 16:1Δ7 and 16:2Δ7,10 fatty acids in Desertifilum is proposed.

Keywords: Desertifilum; molecular phylogeny; polyphasic approach; ultrastructure; fatty acids; fatty acid desaturases

INTRODUCTION

Cyanobacteria are known as producers of many biologically active compounds that may be used for biotechnological or medical purposes. Clearly the search for new bioactive compounds includes both the discovery and thorough analysis of new cyanobacterial strains that can potentially serve as efficient bioproducers. At the present time, unusual extreme environments provide a variety of undescribed microorganisms and are a great source of new strains for cyanobacterial research.

Accurate assessment of a newly isolated cyanobacterial strain is important not only for its individual taxonomic characterization, but contributes to the overall cyanobacterial systematics as well. In this work, we use a polyphasic approach suggested by Komárek and co-authors (Komárek et al. 2014). This approach implies characterization of an organism at morphological, ultrastructural, biochemical and molecular levels and significantly improves the quality of assessment. Current developments in next-generation sequencing allow fast and efficient
characterization of bacterial genomes. Even a draft genome mining is sufficient to estimate the taxonomical position of an organism and evaluate its biotechnological potential.

In this work we present the results of isolation and polyphasic assessment of a new thermophilic cyanobacterial strain of Desertifilum, IPPAS B-1220.

MATERIALS AND METHODS

Collection, purification and growth of the cyanobacterial strain

Samples of cyanobacteria were taken from Lake Shar-Nuur, a freshwater lake in Bayan-Ölgii Aymag (Mongolia), in June 2013, when the temperature of neutral water (pH 6) on the surface was about +35°C. Isolation and cultivation of the IPPAS B-1220 strain had been performed using standard methods. Algologically pure and axenic culture of the non-heterocystous, non-branching filamentous cyanobacterium was obtained via serial dilutions and plating on BG-11 solid medium (Rippka 1988). The culture was maintained in 300 ml Erlenmeyer flasks with 100 ml of BG-11 liquid medium in an orbital shaker at room temperature with cool white luminescent lamps providing an average irradiation of 50 μmol photons m⁻² s⁻¹.

Experimental conditions of cultivation

Intensive cultivation was carried out in a custom-made installation (apparatus), developed in our laboratory, in glass vessels with 250 ml of BG-11 medium. Filaments were grown under continuous illumination with white luminescent lamps at 110 μmol photons m⁻² s⁻¹, and aeration with a sterile air–gas mixture that contained 1.5–2% CO₂. To determine the optimal growth temperature, cultures were incubated at five different temperatures: 28 ± 1°C, 34 ± 1°C, 37 ± 1°C, 40 ± 1°C and 46 ± 1°C. All growth experiments were repeated at least 3 times. For further analysis, cultures were grown at 32 ± 1°C unless otherwise specified.

Growth rate estimation

Growth rate was estimated based on dry biomass accumulation under experimental growth conditions during 3 days. Three aliquots (30–50 ml) of both initial control and experimental cultures were collected by centrifugation, washed with distilled water, dried at 80°C, and weighed.

Light and electron microscopy

The morphology of the strain was accessed by light microscopy (Axio Imager A1; Carl Zeiss, Germany) as described previously (Sarsekeyeva et al. 2014). Morphological characterization and assignment was done as described in Komárek (2006). Transmission electron microscopy was performed using a TEM Libra-120 (Carl Zeiss, Germany) as described in Sarsekeyeva et al. (2014).

Lipid content and fatty acid analysis

Lipid content and fatty acid composition were determined as described earlier (Sarsekeyeva et al. 2014) except that fixed samples were saponified in the presence of heptadecanoic acid as an internal standard with 6% potassium hydroxide solution in 80% ethanol for 1 h at 70°C followed by neutralization of KOH with a 20% solution of sulphuric acid and extraction with hexane of free fatty acids. Double bond position in fatty acids was determined via mass spectrometry of their corresponding 3-pyridylcarbinol esters (Christie and Han, 2010).

DNA isolation and sequencing

Total genomic DNA was isolated from cyanobacterial cells at stationary growth phase according to Campbell and Laudenbach (1995). DNA library preparation and genomic DNA sequencing was performed as described in Mironov et al. (2016). The genome sequence of Desertifilum sp. strain IPPAS B-1220 was deposited at NCBI with the following attributes: SAMN05788062 for BioSample, PRJNA343432 for BioProject and SRR4255595 for SRA database. The genome PGAP file accession number is MJGC00000000.

Bioinformatics

Phylogenetic analysis

The 16S rRNA gene sequence (1482 bp) was found in Desertifilum sp. strain IPPAS B-1220 genome using BLASTn (http://blast.ncbi.nlm.nih.gov; Zhang et al. 2000) and deposited in GenBank under accession number KU556389. The sequence was aligned with nine cyanobacterial 16S rDNA sequences with the highest homology score (identity ≥93%, coverage ≥87%), which were identified using BLASTn. Alignment was performed with ClustalW in MEGA6 (Tamura et al. 2013). The 16S rRNA gene of Gloeobacter violaceus FCC 7421 was used as an outgroup.

Phylogenetic analysis of cyanobacterial 16S rRNA genes was inferred using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei 1993) with 1000 bootstrap repetitions and other default parameters. There was a total of 1270 positions in the final dataset. Pairwise sequence similarity percentages were assessed from p-distance matrices calculated in MEGA6 from nucleotide alignments under pairwise deletion of alignment gaps and missing data among Desertifilum strains.

Analysis of 16S–23S rRNA internal transcribed spacer sequences

The nucleotide sequence of the 16S–23S internal transcribed spacer (ITS) region for Desertifilum sp. strain IPPAS B-1220 was extracted from the partial genomic DNA segment (contig) NZ_MJGC0100009.1, available at the NCBI. Sequences of 16S–23S ITS for other than Desertifilum sp. PPPAS B-1220 organisms were extracted from the corresponding partial sequences of rRNA operons obtained from the NCBI database. GenBank accession numbers of these sequences were: FJ159001 for D. tharense PD2001/TDC17; KM438193 for Desertifilum sp. NapGTcm17, KR269852 for D. tharense A; and KJ028039 for D. fontinalis KR2012/2.

Alignment of the nucleotide sequences was performed using the Clustal W method of Lasergene MegAlign module v. 14.0.0 (DNASTAR Inc., Madison, WI, USA). Both conserved and variable regions of 16S–23S rRNA ITS were identified according to Iteman et al. (2000), Dadheech et al. (2014) and Bravakos et al. (2016). Secondary structures were obtained using Fold software version 2.2 (Ding, Chan and Lawrence 2004), using default settings. Structures with minimal Gibbs energy were chosen for the analysis.

Assignment of genes for fatty acid desaturases

A draft genome sequence of Desertifilum sp. strain IPPAS B-1220 (GenBank accession number NZ_MJGC00000000) was automatically annotated and then manually checked for the presence of genes that encode fatty acid desaturases (FADs). Six candidate genes have been selected and analyzed by BlastP software:
RESULTS AND DISCUSSION
Morphological and structural characterization of the isolated cyanobacterial strain

Axenic culture of a non-heterocystous, non-branching filamentous motile cyanobacterium was isolated from a water sample collected from the freshwater lake, Lake Shar-Nuur, in Bayan-Olgiy Aymag (Mongolia), as described in Materials and Methods. The isolated strain is deposited in the Collection of Microalgae and Cyanobacteria of the Institute of Plant Physiology RAS (Moscow, Russia) under accession number IPPAS B-1220.

While growing in a liquid culture without shaking, the strain exists as a suspension with small clumps (Fig. 1a), forming a thick dense film with increasing age (Fig. 1b). Alternatively, when maintained on agar plates for 2–3 days, the strain appears as filaments spreading out in spiral patterns around the surface (Fig. 1c).

Under light microscope the filaments look green or bright blue-green in color depending on growth conditions. They are thin in shape and long in size (more than 500 μm), either solitary or organized in loose bundles, sometimes forming a dense mesh with intertwined trichomes (Fig. 1d–f). Filaments are motile, non-constricted or slightly constricted at cross-walls, and often attenuated at the ends (Fig. 1h–k). A thin and colorless sheath can be observed only at the ends of some trichomes (Fig. 1g and l). Thick and firm, colorless or brownish slime aggregates are often found between entangled filaments (Fig. 1g). Individual cells in filaments have either barrel, cylindrical, isodiametric or elongated shapes. The cells are usually 2.8 ± 0.3 μm wide and 3.8 ± 1 μm long. Some cells contain inclusions similar to those found in the type strain of D. tharense and identified as gas vesicles by Dadheech et al. (2012) (Fig. 1h and l). Apical cells are circular or conical in shape (Fig. 1h–l), sometimes with extrusions (Fig. 1m).

The absorption spectrum of the isolated IPPAS B-1220 strain suspension is typical of cyanobacteria and consistent with the presence of chlorophyll a, carotenoids, allophycocyanin and phycocyanin (Fig. S1 in the online supplementary material).

Ultrastructural analysis suggests that cells are circular in cross-section (Fig. 2a and d) as well as rectangular in longitudinal section (Fig. 2a and b), and of a different length. Thylakoids are parietal; frequently a bundle or several bundles of 2–5 thylakoids are observed across a cell. Sometimes, the thylakoid bundles are observed to form concentric circles (Fig. 2a–e) with a carboxysome located in the center (Fig. 2b–d).

Several carboxysomes are usually present in each section of the cell (Fig. 2b and d). Besides carboxysomes, other inclusion
Figure 2. Electron microphotographs of the isolated cyanobacterial strain cells. (a) General view of filament parts, cross and longitudinal sections; (b) higher magnification of the longitudinal section of a filament part; (c) magnified longitudinal section with concentric circles formed by thylakoids surrounding carboxysome; (d) cross section of a filament; (e) oblique section of a filament surrounded by sheath. C: carboxysomes, Cy: cyanophycin; M: cell membrane; O: outer membrane; P: peptidoglycan; S: S-layer; Sh: sheath; asterisks indicate inclusions with low electron density, probably gas vacuoles; white arrows mark osmiophilic inclusions, large black arrows mark concentric circles formed by thylakoids inside cell; small black arrows mark excreted exopolysaccharides.

bodies are clearly seen: in addition to cyanophycin (Fig. 2a), distinct small osmiophilic inclusions are found to be often located along cross-walls (Fig. 2a, b and e). Another type of observed inclusions has a low electron density (Fig. 2a, b and e) comparable to those previously identified in D. tharense gas vacuoles (Dadheech et al. 2012).

On the outer membrane of cells, we were able to identify an extra layer (S-layer) (Fig. 2c). We also detected a fibrous sheath preserved around certain cells (Fig. 2d and e), and all cells were noted to excrete exopolysaccharides in the form of small droplets (Fig. 2a–e).

According to our observations, the isolated cyanobacterial strain is comparable to the type strain D. tharense (Dadheech et al. 2012), except for the extrusions at its apices (Fig. 1m), which were previously described only in D. fontinale (Dadheech et al. 2014).

Phylogenetic analysis
The 16S rDNA nucleotide sequence of IPPAS B-1220 displays 99.9% pairwise sequence similarity values with several strains, such as the type strain of D. tharense from Indian desert soil (Dadheech et al. 2012), D. tharense A from a lake in China (GenBank, KR269853) and a hot spring strain from Greece, Desertifilum sp. NapGTCm17 (Bravakos et al. 2016) (Table S1 in the online supplementary material). Lower similarity was found between IPPAS B-1220 and D. fontinale KR2012/2 isolated from a Kenyan warm spring (Dadheech et al. 2014), as well as a freshwater strain Desertifilum sp. RBD02 from the Ganges river (India) (GenBank, KT445937) (98.8% and 96.5%, respectively). All these strains except for Desertifilum sp. RBD02 have at least 98.8% or higher similarity between their 16S rDNA sequences and thus definitely belong to the same genus.
A maximum likelihood phylogenetic tree was constructed for IPPAS B-1220 using the best BLAST hits for its 16S rDNA sequence (Fig. 3a). Apparently, IPPAS B-1220 belongs to the subclade that includes the type strain D. tharense PD2001/TDC17, as well as two other Desertifilum strains. Noticeably, Desertifilum sp. RBD02 is positioned as a separate lineage, sister to all mentioned strains, and D. fontinale forms its own clade distinct from all other Desertifilum strains.

The cluster made of all Desertifilum strains appears to be separated with the high bootstrap support from the sister clade strains identified as Oscillatoria tenus and Coleofasciculus chthonoplastes (formerly, Micrococus chthonoplastes), to which they have only around 93–95% identity in 16S rRNA gene sequences.

Based on the 16S rDNA-based phylogeny, we can conclude that the newly described strain is a close relative to the type strain D. tharense PD2001/TDC17. However, we confirm that it is not identical to the latter.

The sequences of 16S–23S rDNA ITS regions of Desertifilum strains show high similarity (Fig. S2 in the online supplementary material). Only single nucleotide differences between analysed strains are found in sequences of box B and V3; D1–D1′ and V2 helices appear to be more variable. Other conserved domains of ITS are identical in all strains (Fig. S2).

Secondary structures of D1–D1′ and V2 helices were modeled for all analyzed strains (Fig. 3b and c). In all studied Desertifilum strains the conservative sequence of the D1 region GACC is repeated two times, and these repeats are separated by one nucleotide (Fig. S2). Only the second GACC repeat forms the stem of a helix and thus we counted it as the beginning of the D1–D1′ hairpin. All D1–D1′ helices of studied Desertifilum strains have short stems consisting of four nucleotides, internal and terminal loops and a side hairpin terminated by a loop (Fig. 3b). The side-loop forming the second hairpin is a feature characteristic only of Desertifilum strains.

According to our data, Desertifilum strains NapGTcm17 and D. tharense A are very similar to the type strain PD2001/TDC17 and, most probably, represent the same species of D. tharense (Fig. S2 in the online supplementary material and Fig. 3). Desertifilum fontinale is the most divergent from PD2001/TDC17 and, undoubtedly, represents a separate species. This result is in complete agreement with Dadheech et al. (2014) and Bravakos et al. (2016). The isolated strain IPPAS B-1220 is in-between D. tharense and D. fontinale: it is more similar to the type strain of D. tharense than to D. fontinale, but more distant from the type strain than both strains NapGTcm17 and D. tharense A.

In conclusion, IPPAS B-1220 definitely belongs to the newly described genus Desertifilum (Dadheech et al. 2012) distributed in China, Mongolia, India, Greece and Kenya. The genus inhabits both warm freshwater reservoirs and desert soils. Despite its wide distribution the genus remains genetically fairly stable. High genetic similarity makes species identification problematic, and the position of IPPAS B-1220 at the species level remains questionable. Therefore, more data on morphology, biochemistry and physiology, as well as the complete genome sequence of other Desertifilum strains are necessary for the accurate strain assessment at the species level.

Growth characteristics

Similarly to other thermotolerant strains of the same genus, Desertifilum sp. IPPAS B-1220 grows well with a doubling time of 12–14 h at temperatures ranging from 28 to 40°C. No growth was observed at 46°C. An earlier described strain of Desertifilum PD2001/TDC17 (Dadheech et al. 2012) grew well in a similar range of temperatures. However, it had a distinct growth optimum at 30–35°C and grew two times slower in comparison with Desertifilum sp. strain IPPAS B-1220. The enhanced growth rate of the latter was probably due to a higher light intensity and CO₂ supplement (up to 2%). The pronounced thermotolerance and the fast growth rate are valuable features for various biotechnological applications of this strain.

Fatty acid composition

Analysis of the fatty acid (FA) composition of total lipids is a useful complement to other approaches in polyphasic assessment of cyanobacteria (Kenyon and Stanier 1970; Murata et al. 1992; Los and Mironov 2015). Information on the FA composition of Desertifilum strains may facilitate the identification of new species in this genus and a more precise classification of strains. It should also be noted that many Desertifilum strains are thermo-tolerant extremophiles that can adapt to a wide range of ambient temperature. The extent of FA desaturation is an important factor for temperature acclimation (Sinetova and Los 2016).

Cells of Desertifilum sp. grown at 37°C were found to accumulate up to 40–60 mg of FAs per gram of dry weight. FA composition was 37% of saturated and 63% of unsaturated FAs with an unsaturation index of 1.0 (Table 1). One unique feature of Desertifilum sp. strain IPPAS B-1220 is the ability to accumulate a significant amount (28–40% of all FAs) of hexadecadienoic acid, 16:2Δ7,10.

Hexadecadienoic acid was identified by National Institute of Standards and Technology Mass Spectrometry (NIST MS) http://chemdata.nist.gov/mass-spc/ms-search/ search software as 7,10- or 9,12-methyl hexadecadienoate in a mass spectrum of a peak with retention time (RT) of 18.12 min (Fig. 4a, equal similarity to library records of ~99%). Such uncertainty in identification can be explained by a known fact that methyl esters of FAs with clearly resolved peaks, different RT and relative RT values sometimes display very similar mass-spectral data (Spitzer 1997).

To identify the exact position of double bonds in this FA, we analyzed its 3-pyridylcarbinol ester by mass spectrometry (Fig. 4b). We identified a molecular ion with m/z = 343 suggesting hexadecadienoic acid. Ions that correspond to cleavage on either side of the terminal double bond were detected at m/z = 272 and 246 (26 atomic mass units (amu) apart); then there was a gap of 14 amu to m/z = 232, and then a gap of 26 amu for the internal double bond to m/z = 206. In addition, there were two gaps of 40 amu for the double bond and associated methylene group on the carboxyl side: between m/z = 286 and 246, and between 246 and 206. Starting downstream from m/z = 206 to the prominent ions with m/z = 164, 151, 108 and 92 (fragments located close to a pyridine ring), we identified regular series of ions 14 amu apart to the side of the double bond. This confirmed that there were no further functional groups in this region. The fragmentation pattern observed indicates that the fatty acid can be identified as exactly 7,10-hexadecadienoic acid, 16:2Δ7,10.

Among cyanobacteria, hexadecadienioic FAs have been found in some strains of Nostoc (Holten, Blecker and Stevens 1968; Salil, Nimer and Radwan 1990), Anabaena (Li and Watanabe 2001), Spirulina subsalsa and Plankothrix agarthii NIES-204 (Li et al. 1998), Tolyphorix sp. (Maslova et al. 2004), Oscillatoria willei (Parker et al. 1967), Chamaesiphon minutus PCC 6605, Cyanothecae sp. PCC 6910 (Kenyon 1972), and Halothecae sp. PCC 7418 (Oren et al. 1985). However, the exact positions of double bonds were not specifically determined. Hexadecadienoic acid with the double bonds at positions Δ7 and Δ10 was first identified in a fraction of
Figure 3. Phylogenetic relationships of Desertifilum strains based on homology of 16S rRNA genes and analyses of secondary structure of 16S–23S rRNA ITS regions. (a) Maximum likelihood phylogenetic tree is based on 16S rRNA gene sequences that produced highest homology score with the sequence of 16S rDNA of the isolated Desertifilum IPPAS B-1220 strain and with Gloeobacter violaceus PCC 7421 as an outgroup. Bootstrap values >50% are shown above branches. (b) and (c) Secondary structures of 16S–23S ITS regions: D1–D1′ helices (b) and V2 helices (c). (1) Desertifilum sp. strain IPPAS B-1220. (2) Desertifilum tharense PD2001/TDC17 (Dadheech et al. 2012). (3) Desertifilum sp. NapGTcm17 (Bravakos et al. 2016). (4) Desertifilum tharense A, a strain isolated in China (GenBank, KR269852). (5) Desertifilum fontinale KR2012/2 (Dadheech et al. 2014). Nucleotides different from those in sequences of the type strain PD2001/TDC17 are outlined with squares.
Table 1. Fatty acid composition of total lipids of Desertifilum sp. strain IPPAS B-1220 grown at 37° and 23° C.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>37°C (mean ± SD, mass %)</th>
<th>23°C (mean ± SD, mass %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>16:0</td>
<td>35.0 ± 1.5</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>16:1Δ⁷</td>
<td>2.9 ± 0.7</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>16:1Δ⁹</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>16:2Δ⁷,10</td>
<td>28.0 ± 6.8</td>
<td>40.0 ± 4.0</td>
</tr>
<tr>
<td>18:0</td>
<td>2.0 ± 0.4</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>18:1Δ⁹</td>
<td>5.5 ± 0.5</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>18:1Δ₁₁</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>18:2Δ⁸,₁₂</td>
<td>24.0 ± 1.5</td>
<td>26.0 ± 1.5</td>
</tr>
<tr>
<td>UI</td>
<td>1.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

¹Mean percentage from three replicates. SD: standard deviation of the replicates. UI: unsaturation index (relative units).

Cyanobacteria have been divided into four large groups according to both FA composition of their membrane lipids (Murata et al. 1992) and the presence of genes for FADs in cyanobacterial genomes (Los and Mironov 2015).

Group 1 consists of strains that possess one or several (usually, two) genes for the acyl-lipid fatty acid desaturases (FADs), namely, Δ⁹-desaturases. These strains synthesize only mono-unsaturated FAs with a double bond at the Δ⁹ position. Strains of Group 2 (mainly marine cyanobacteria) possess Δ⁹- and Δ₁₂-FADs and synthesize FAs with two double bonds at positions Δ⁹ and Δ₁₂. Group 3 is characterized by the presence of three FADs, either Δ⁹-, Δ₁₂- and Δ₁₆-FADs, or Δ⁹-, Δ₁₂- and Δ₁₆,FADs. These strains are capable of synthesizing trienoic FAs (ω- or ω₁-linolenic acids, respectively). Cyanobacteria of Group 4 express all four FADs, Δ⁹-, Δ₁₂-, Δ₁₆- and Δ₁₈-desaturases, and may synthesize tetraenoic stearidonic acid, 18:4. So far, no other FADs have been reported in cyanobacteria. The exception is Prochlorothrix hollandica, which, in addition to two regular Δ⁹-FADs, DesC1 and DesC2, possesses an unusual Δ₄-FAD activity (Gombos and Murata 1991). The latter enzyme, however, has not been biochemically characterized.

According to this classification, Desertifilum sp. IPPAS B-1220 belongs to Group 2 that produces mono-unsaturated and di-unsaturated FAs. However, in addition to the ordinary cyanobacterial FAs desaturated at positions Δ⁹ and Δ₁₂, IPPAS B-1220 produces monoenic 16:1Δ⁵ and dienoic 16:2Δ⁷,₁₀ fatty acids.

Our previous analysis of the draft genomic sequence of Desertifilum (Mironov et al. 2016) revealed the presence of six genes for acyl-lipid desaturases: two genes for Δ⁹-FADs, desC1 (WP_069969815) and desC2 (WP_069969842); two Δ₁₂-FADs, desA1 (WP_069965515) and desA2 (WP_069966365); and two genes for FADs of unknown specificity, desX (WP_069966662) and desG (WP_069966365). Two genes for Δ⁹-FADs and two genes for Δ₁₂-FADs, which are highly identical to the corresponding genes of other cyanobacterial strains, have been annotated using a standard homology search (Sakamoto et al. 1994, Los and Mironov 2015). A gene for the desaturase of unknown function and specificity, desX (all1611 in Synechocystis sp. strain PCC 6803), was described earlier (Sinetova and Los 2016). This gene also has a high degree of similarity to other cyanobacterial homologs and was annotated with confidence.

The gene of the sixth FAD (WP_069966365) of Desertifilum, which we named DesG, was automatically identified as encoding a Δ₁₂- or ω₃-FAD (Mironov et al. 2016). However, it is only 19% identical and 34% similar to the known cyanobacterial genes for Δ₁₂-FADs. Its homology to the ω₃-FADs gene family is even lower. In our opinion, DesG belongs to a specific group of eubacterial FADs, one of them being Δ₅-FAD of Bacillus cereus (Chazarez-Cifre et al. 2013) that is 32% identical and 50% similar to DesG. Genes that encode proteins similar to DesG form a distinct cluster and are widespread in cyanobacteria (Fig. S3 in the online supplementary material). We suggest that this new type of cyanobacterial desaturases may function as a ω₉- or Δ₇-FAD (Fig. 5). If so, the results of fatty acid analysis imply that such an enzyme is specific to palmitic (16:0) acid, and its activity results in the formation of 16:1Δ⁷, which may be further desaturated by Δ₁₂-FAD (which recognizes the third carbon after the first double bond) to produce 16:2Δ⁷,₁₀. This hypothesis, however, requires experimental confirmation of the specific activity of the DesG enzyme and the further action of Δ₁₂-FAD.

Fatty acid desaturases

In cyanobacteria, FA desaturation is induced by low temperatures (Sinetova and Los 2016). FA desaturation is a mechanism to maintain proper membrane fluidity and integrity under cold stress (Allakhverdiev et al. 2009). We investigated changes in FA composition of total lipids isolated from strain IPPAS B-1220 initially grown at 37°C and then exposed to 23°C for 24 h (Table 1). A decrease in ambient temperature caused a decrease in the proportion of saturated FAs in favor of unsaturated FAs. The most noticeable changes were observed in the amounts of 16:0 (a decrease from 35% to 23%) and 16:2Δ⁷,₁₀ (an increase from 28% to 40%) FAs. The amount of 18:2Δ⁹,₁₂ increased from 24% to 26%. The unsaturation index rose from 1.0 to 1.3. Thus, Desertifilum exhibited the general tendency of FA unsaturation at low temperatures. In contrast to ‘model strains’ of cyanobacteria (Synechocystis sp. strain PCC 6803 or Anabaena variabilis) that desaturate FAs at positions Δ₁₂ and/or Δ₁₅ while exposed to cold (Sinetova and Los 2016), Desertifilum desaturates FAs at positions Δ⁷ and Δ₁₀ (Table 1).
Figure 4. Total ion current chromatogram of fatty acid methyl esters from total lipids of Desertifilum sp. strain IPPAS B-1220 (a) and mass spectra of 3-pyridylcarbinol ester of hexadecadienoic acid (b) IS - internal standard.
CONCLUSION

Here we characterized a new cyanobacterial strain, *Desertifilum* sp. IPPAS B-1220, with an unusual fatty acid composition. We have utilized a polyphasic approach developed for the assessment of newly discovered cyanobacterial strains. The approach employed morphological, biochemical and molecular analysis on the whole-genome scale, which provided the basis for an accurate systematic annotation and insights into the lipid metabolism of the strain.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLG online.

FUNDING

This work was supported by a grant from the Russian Science Foundation [no. 14-14-00904] to MAS, and by a grant from the Ministry of Education and Science of the Republic of Kazakhstan to BKZ [no. 1582/GF4].

Conflict of interest. None declared.

REFERENCES


Dadheech PK, Mahmoud H, Kotut K et al. *Desertifilum fontinale* sp. nov. (Oscillatoriales, Cyanobacteria) from a warm spring in East Africa, based on conventional and molecular studies. Fottea 2014;14:129–40.


Spitzer V. Structure analysis of fatty acids by gas chromatography—low resolution electron impact mass spectrometry of their 4,4-dimethyloxazoline derivatives—a review. Prog Lipid Res 1997;35:387–408.