**Methylobrevis albus** sp. nov., isolated from freshwater lake sediment

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**Abstract**

An aerobic, Gram-stain-negative, rod-shaped bacterium with flagellum, designated **L22**<sup>T</sup>, was isolated from sediment of Hulun Lake, Inner Mongolia, China. The organism was found to grow optimally at 30°C in a medium containing 0–0.75% (w/v) NaCl at pH 7.5. The major fatty acid identified was summed feature 8 (C<sub>18:1</sub>ω<sub>7c</sub>). The dominant polar lipids were phosphomonoester, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. The main respiratory quinone was Q-10. The draft genome sequence of strain **L22**<sup>T</sup> consisted of 4354,788 bp. The G+C content of genomic DNA was 69.8 mol %. The 16S rRNA gene sequences indicated that strain **L22**<sup>T</sup> was affiliated with the genus *Methylobrevis* within the family **Pleomorphomonadaceae**, being most closely related to *Methylobrevis pamukkalensis* JCM 30229<sup>T</sup> with 95.9% 16S rRNA gene sequences similarity. The AAI, ANI and dDDH values between strain **L22**<sup>T</sup> and *M. pamukkalensis* JCM 30229<sup>T</sup> were 72.5%, 80.7% and 22.7%. Based on taxonomic results in this study, we proposed that strain **L22**<sup>T</sup> a novel species in the genus *Methylobrevis* of the family **Pleomorphomonadaceae**, for which the name *Methylobrevis albus* sp. nov. is proposed. The type strain is **L22**<sup>T</sup> (=KCTC 72858<sup>T</sup>=MCCC 1H00432<sup>T</sup>).

**Keywords** *Methylobrevis albus* · **Pleomorphomonadaceae** · 16S rRNA gene · Polyphasic taxonomy

**Abbreviations**

*KCTC* · Korean Collection for Type Cultures  
*MCCC* · Marine Culture Collection of China  
*MEGA* · Molecular Evolutionary Genetics Analysis  
*HPLC* · High-performance liquid chromatography  
*TLC* · Thin-layer chromatography  
*JCM* · Japan Collection of Microorganisms

**Introduction**

The genus *Methylobrevis* was first described by Poroshina et al. (2015) as a member of the order **Rhizobiales** within the class **Alphaproteobacteria**. Up to now only one described species, *M. pamukkalensis* (Poroshina et al. 2015), has been confirmed to belong to the genus *Methylobrevis* within the family **Pleomorphomonadaceae**. The family **Pleomorphomonadaceae** was recently revised by transferring the genus *Chthonobacter* (Kim et al. 2017), *Hartmannibacter* (Suarez et al. 2014), *Methylobrevis* (Poroshina et al. 2015), *Mongoliimonas* (Xi et al. 2017), *Oharaeibacter* (Lv et al. 2017) and *Pleomorphomonas* (Xie and Yokota 2005) into the family **Pleomorphomonadaceae** (Hördt et al. 2020). In October 2019, we isolated the strain **L22**<sup>T</sup> from sediment of Hulun Lake, Inner Mongolia, China. Based on the phenotypic, genotypic and chemotaxonomic characteristics, we aimed to propose a novel species of the genus *Methylobrevis* within the newly identified family **Pleomorphomonadaceae**, represented by strain **L22**<sup>T</sup>.

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The GenBank accession number for the 16S rRNA gene sequence of *Methylobrevis albus* **L22**<sup>T</sup> is MW195049 and the draft genome has been deposited in GenBank under the accession number JADZLT000000000.
Materials and methods

Inoculum source, isolation and preservation

Sample of fresh water and sediment was collected from the middle part of Hulun Lake (117°01’ 10” E, 48°30’ 40” N), Inner Mongolia, the fourth biggest freshwater lake in China in July 2019. The sediment sample was diluted serially to 10⁻³ with sterile water. 0.1 ml aliquots of each dilution were spread onto 1/2-strength R2A agar. The R2A agar was made with 0.05% tryptone, 0.05% yeast, 0.05% casein, 0.05% starch, 0.05% glucose, 0.03% sodium pyruvate, 0.03% K2HPO4 and 0.005% MgSO4•7H2O, all w/v. The pH was adjusted to pH 7.2. After incubating in an aerobic environment at 28° C for 15 days, strain L22T was picked among several tiny white colonies and purified on R2A agar by routinely repetitious streaking. The strain was stored in sterile 15% (v/v) glycerol supplemented with 1% (w/v) NaCl suspensions at −80° C. Strain L22T was cultivated on R2A agar at 30° C unless otherwise mentioned. For comparison and analysis, we used Methylobrevis pamukkalensis ICM 30229T as a related strain, which was cultured under the same conditions as strain L22T.

16S rRNA gene sequence analysis

Universal bacterial primers 27F and 1492R as previously described (Liu et al. 2014) were used for 16S rRNA gene sequence PCR amplification. All PCR production was purified and ligated into the PMD-18 vector (Takara). Cloned Escherichia coli DH5α cells (Trans-Gen Biotech) were then transformed with the obtained recombinant plasmids. The positive clones were selected by LB agar containing 0.01% (w/v) ampicillin and sequenced at BGI Co. Ltd (Qingdao, China) using the ABI 3730XL system. The almost complete 16S rRNA gene sequence of strain L22T was compared with those available from the EzTaxon server (http://www.ezbiocloud.net/identify) (Kim et al. 2012) and NCBI database (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) for further phylogenetic analysis. Sequences of the related type strains and the almost completed 16S rRNA gene sequence of strain L22T were aligned using MUSCLE (Robert and Edgar 2004). A phylogenetic tree was reconstructed utilizing the maximum-likelihood (ML) algorithm (Felsenstein 1981) implemented in the software package MEGA 7.0 (www.megasoftware.net) (Sudhir et al. 2016). The overall mean distance of aligned 16S rRNA sequences was calculated and showed suitable compatibility, thus a neighbour-joining (NJ) (Saitou and Nei 1987) tree was reconstructed to verify the confidence of the ML tree. For all tree-making calculations, variance estimates were taken on for 1000 replication by the method of bootstrap analysis to ensure accurate calculation results.

Genome sequence analysis

The genomic DNA of strain L22T was extracted and purified using a bacterial genomic DNA kit (Takara). The draft genomic sequence of strain L22T was sent to Shanghai OE Biotech Co., Ltd (Shanghai, China) and sequenced using Illumina technology. An Illumina shotgun library using the Illumina TruSeq Nano DNA Sample Prep Kit was reconstructed and sequenced using the pair-end 350-bp protocol on the Illumina HiSeq Xten platform (Illumina Inc., San Diego, USA). Raw sequencing data were generated by Illumina base-calling software CASAVA v1.8.2 (http://support.illumina.com/) according to its corresponding manuscript. The sequenced reads were assembled using SOAP de novo software (Li et al. 2009). Gene content was annotated using the NCBI Prokaryotic Genome Annotation Pipeline and the genes involved in metabolic pathways were analysed in detail by using information from the KEGG database (Kanehisa et al. 2016). Protein-encoding regions were identified with the Rapid Annotations using Subsystem Technology (RAST) server (Aziz et al. 2008). A further phylogenetic and taxonomic multilocus sequence analysis was made to identify the accurate taxonomic status of strain L22T. Genomes of the order Hyphomicrobiales which were available from the NCBI were aligned with MUSCLE v.3.8.31. The phylogenomic relationship was analysed by Genome Taxonomy Database (GTDB; https://gtdb.ecogenomic.org), and the phylogenetic trees were constructed by using FastTree (Luke 2002) using GTR+CAT parameters and IQTree (Trifinopoulos et al. 2016) using GTR+F+I+G4 model and 1000 bootstrap replicates. The genome of Ochrobactrum lupini ATCC 49188T served as the outgroup to compute the phylogeny for the DNA and protein sequences. The tree was collapsed and formatted using iTOL v4 (Ivica et al. 2019).

The average amino acid identity (AAI) values between genomes of strain L22T and the closely related members of the family Pleoromophomonadaceae were calculated by CompareM (https://github.com/dparks1134/CompareM). Digital DNA–DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC 2.1; http://ggdc.dsmz.de). The orthologous average nucleotide identity (ANI) values were calculated using an online server (http://enve-omics.ce.gatech.edu/ani/) (Chun et al. 2015).

Morphological, physiological and biochemical analysis

Morphological features of strain L22T were tested using the biomass incubated on R2A agar at 30 °C for 3 days.
Light microscopy (E600; Nikon) and transmission electron microscopy (JEM1200, Japan) were used to observe the microstructures of cells of strain L22T. The motility was tested using the hanging-drop method and gliding motility was confirmed as described by Bowman (Bowman 2000). Gram-staining was performed using a Gram stain kit (bioMérieux) according to the manufacturer’s instructions. Different gradients of 0, 4, 10, 15, 20, 25, 28, 30, 33, 37 and 40 °C were set up to find the temperature range for growth on R2A agar. The tolerances to various NaCl concentrations were examined by incubating strain L22T on R2A agar where 0.00–10.00% (w/v) NaCl at intervals of 0.25% (w/v) was added. The pH range for growth was determined by a series of adjusted R2A broth adding a series of 20 mM buffer: MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5), CAPSO (pH 9.0, 9.5 and 10.0) and CAPS (pH 10.5 and 11.0). Growth at an anaerobic (10% H2, 10% CO2 and 80% N2) environment was confirmed after 14 days of incubation on R2A agar with or without 0.1% (w/v) KNO3 in an anaerobic bag. All experiments were performed with three replicates. The reduction of nitrate was tested following a previous statement of Cowan and Steel (Cowan and Steel 1974). Oxidase activity was tested using an oxidase reagent kit (bioMérieux) according to the manufacturer’s instructions. Catalase activity was determined by the application of 3% (v/v) hydrogen peroxide solution and observing bubble production. Tests for the hydrolysis of alginate, carboxymethylcellulose, starch, casein, Tweens 20, 40, 60 and 80 were performed as previously mentioned (Cowan et al. 1966). DNase activity was investigated by DNase agar (HopeBio) according to the manufacturer’s instruction. Sensibility to antibiotics was investigated on R2A agar using the disc diffusion method following the procedures outlined by the Clinical and Laboratory Standards Institute (CLSI 2018). Methanol utilization was tested in 500 ml flasks containing 100 ml of sterile marine ammonium mineral salt (MAMS) medium supplemented with 2% (v/v) methanol and 0.4 ml of a vitamin mixture (thiamin, biotin, folic acid and B12, 50 μg l–1 of each). The MAMS medium (Schäfer, 2007) contained (w/v): NaCl, 2%; K2HPO4, 0.234%; (NH4)2SO4, 0.1%; MgSO4·7H2O, 0.1%; KH2PO4, 0.036%; CaCl2, 0.015%; Na2MoO4·2H2O, 0.002%; Na2WO4·2H2O, 0.0003%; FeSO4·7H2O, 0.0002%. The pH was adjusted to pH 7.0. Same flasks with no methanol were set as a blank control group. All flasks were incubated for one week at 30 °C with shaking (100 rpm). Strain L22T and strain M. pamukkalensis JCM 30229T were treated in the same way. Activities of other enzymes were evaluated in virtue of API ZYM (bioMérieux) kits according to the manufacturer’s instruction. Oxidation of various carbons as the sole carbon source was assessed in Biolog GEN III microplates (http://www.biolog.com/) according to the manufacturer’s instruction. Acid production of different compounds as sole sources of carbon and energy was determined using API 50CHB (bioMérieux) kits according to the manufacturer’s instruction. Other biochemical tests were performed with the API 20E (bioMérieux) kits according to the manufacturer’s instruction. Each one of those API and Biolog tests was carried out using the biomass of strain L22T grown on R2A agar at 30 °C for 3 days and repeated for two times simultaneously with the related type strain.

### Chemotaxonomic characterization

Late-exponential-growth-phase biomass of strain L22T and its relative grew on R2A agar at 30° C were collected in centrifuge tubes and impermanently preserved at −20° C ready for imminent investigations. The cellular fatty acid methyl esters were prepared and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1) (Athalye et al. 2010). The subsequent identification was performed by an Agilent 6890 N gas chromatography basing on the TSBA40 database of the microbial identification system. Polar lipids were obtained by a series of centrifugation and extraction and separated via TLC (thin layer chromatography) as described previously (Minnikin, 1984). The respiratory quinone was extracted from 300 mg of freeze-drying biomass and analysed using reverse-phase HPLC (high-performance liquid chromatography), according to the method used previously (Minnikin et al. 1984).

### Results and discussion

#### 16S rRNA gene sequence and phylogenetic analysis

The almost complete 16S rRNA (1457 bp) gene sequence of strain L22T was obtained through conventional Sanger sequencing. The result of pairwise comparison of 16S rRNA gene sequences in the GenBank database of NCBI server (https://www.ncbi.nlm.nih.gov) showed that the most related strain was Methylobrevis pamukkalensis JCM 30229T with 95.9% 16S rRNA gene sequence similarity, followed by Chthonobacter albigriseus JCM 30603T (95.0%), Ohraraeibacter diazotrophicus SM30T (94.5%), Nitratireductor lucknowense IITR-21T (93.8%) and Nitratireductor indicus C115T (93.8%). The 16S rRNA gene sequence of strain L22T showed a maximum identity of 95.9% with validly published species, which is below the 98.7% species threshold identity but higher than the 94.5% genus threshold identity. Following the 16S rRNA-based taxonomic identity, the species rank thus seems appropriate for this strain. Besides, the phylogenetic maximum-likelihood tree based on 16S rRNA gene sequences (Fig. 1) showed that strain L22T belonged to the genus Methylobrevis. The topology of the phylogenetic tree constructed by neighbour-joining arithmetic (Fig. S1)
proved the conclusion that strain L22T and M. pamukkalensis JCM 30229T formed a stable cluster that had a distinct phylogenetic lineage with other genera within the family Pleomorphomonadaceae. These overall data indicated that strain L22T was regarded as a novel species within the genus Methylobrevis.

**Genome sequence analysis**

The draft genome sequence of strain L22T consisted of 4,354,788 bp. The combined assembly yielded 60 contigs with the largest being 618,762 bp and the contig N50 value was 249,401 bp. An average 473×coverage depth was accomplished. The DNA G+C content of strain L22T was 69.8 mol%, which was following the reported value for strain M. pamukkalensis JCM 30229T (67.9 mol%) (Poroshina et al. 2015). Gene prediction and annotation identified 3927 genes, 3866 protein-encoding genes, 51 tRNA genes, 6 rRNA genes. Two 16S rRNA gene sequences (1167 bp and 1473 bp) was extracted from the draft genome and were compared with the almost complete 16S rRNA (1457 bp) gene sequence of strain L22T obtained through conventional Sanger sequencing to ensure the authenticity. Moreover, according to annotation from KEGG database, genes assigned to functional categories are involved in the environmental information processing (318), carbohydrate metabolism (250), protein families: genetic information processing (227), protein families: signalling and cellular processing (222), genetic information processing (164) and amino acid metabolism (147). Gene katE (encoding catalase) and katE-intracellular protease were found which meant strain L22T got the ability to decompose hydrogen peroxide. A complete phosphatidate cytidylyltransferase [EC 2.7.7.41] pathway and gene pmtA were proved to exist and thus phosphatidylethanolamine (PE) can be produced and transformed into phosphatidylcholine (PC), in accord with the result of polar lipids experiment. Results on KEGG told that strain L22T can reduce sulfate to APS, PAPS, sulfite and finally sulfide step by step. However, the experiment showed that sodium thiosulfate could not be reduced to H2S. Though the existence of gene xoxF, mdh1 and mxaF indicated that methanol could be oxidized into formaldehyde, strain L22T was not capable of methylotrophic growth in the absence of formaldehyde dehydrogenase and formate dehydrogenase. Phylogenetic analysis of the genomic amino acid sequences performed with the IQTree and FastTree approaches demonstrates that strain L22T formed a stable lineage within the genus Methylobrevis (Figs. 2, S2).

The AAI value between strain L22T and M. pamukkalensis JCM 30229T was 72.5%, significantly below the proposed cut-off for a species boundary of 85–90% and exceed the threshold value for a genus boundary 55–60% (Rodriguez-R and Konstantinidis 2014). The results of ANI and dDDH between strain L22T and M. pamukkalensis JCM 30229T were 80.7% and 22.7%, both below the thresholds (ANI: 95–96%; dDDH: 70%) for new species identification (Goris et al. 2007) (Meier-Kolthoff et al. 2013). Details of AAI, ANI and dDDH values between
strain L22\(^T\) and the closely related members of the family \textit{Pleomorphomonadaceae} are listed in Table 1. These results supported the finding that strain L22\(^T\) was a novel member of the genus \textit{Methylobrevis}.

### Table 1 The AAI, ANI and dDDH values between strain L22\(^T\) and the closely related members of the family \textit{Pleomorphomonadaceae}

| Species                           | AAI   (%) | ANI   (%) | dDDH  (%) |
|----------------------------------|---------|----------|----------|
| \textit{Chthonobacter albigriseus} JCM 30603\(^T\) | 67.8    | 78.7     | 21.1     |
| \textit{Hartmannibacter diazotrophicus} KACC 17263\(^T\) | 69.8    | 79.9     | 21.8     |
| \textit{Methylobrevis pamukkalensis} JCM 30229\(^T\) | 72.5    | 80.7     | 22.7     |
| \textit{Mongoliimonas terrestris} MIMtkB18\(^T\) | 68.0    | 78.6     | 21.0     |
| \textit{Oharaebacter diazotrophicus} SM30\(^T\) | 67.6    | 79.1     | 21.0     |
| \textit{Pleomorphomonas oryzae} ATCC BAA-940\(^T\) | 64.7    | 77.5     | 20.3     |

### Morphological, physiological and biochemical characteristics

Strain L22\(^T\) was susceptible to chloramphenicol (30 μg), ofloxacin (5 μg), norfloxacin (10 μg), ceftriaxone (30 μg), tetracycline (30 μg), cefotaxime (30 μg), penicillin (10 μg) and ampicillin (10 μg), resistant to clarithromycin (15 μg), rifampicin (5 μg), gentamicin (10 μg), vancomycin (30 μg), erythromycin (15 μg), streptomycin (10 μg), lincomycin (2 μg), neomycin (30 μg) and tobramycin (10 μg). Like the other strain of the genus \textit{Methylobrevis}, strain L22\(^T\) comprised Gram-stain-negative, strictly aerobic and heterotrophic bacteria that do not form endospores or prokaryote. Cells of the genus \textit{Methylobrevis} are halotolerant restricted facultative methylotrophs that assimilating C1 units via the isocitrate-lyase-positive variant of the serine pathway and accumulating intracellular granules of PHB. However,
differences remained that strain *M. pamukkalensis JCM 30229<sup>T</sup> showed positive for assimilating methanol while strain *L22<sup>T</sup> did not. Its morphological, physiological, and biochemical characteristics that differentiate strain *L22<sup>T</sup> from its related type strain are provided in Table 2. Other properties are available in species description and the negative traits of commercial kits (API ZYM, API 20E, API 50CH and Biolog GEN III) are listed in Table S1.

### Table 2 Characteristics that differentiate strain *L22<sup>T</sup>* and related type strain

| Characteristic                          | 1         | 2         |
|----------------------------------------|-----------|-----------|
| Cell shape and size (μm)               | Short rod | Short rod<sup>a</sup> |
|                                        | 0.3–0.4×0.9–1.1 | 0.7–1×1.5–2<sup>a</sup> |
| Colony colour                          | Milky white | Pale brown<sup>a</sup> |
| Growth at/in NaCl (optimum) (%)        | 0–4.50 (0–0.75) | 0–5.8 (0.5)<sup>a</sup> |
| (pH (optimum))                         | 6.0–9.5 (7.5) | 6.0–9.0 (7.5–8.0)<sup>a</sup> |
| Hydrolysis of Starch                   | −         | +         |
| Enzyme activities                      |           |           |
| Valine arylamidase                     | w         | +         |
| Cystine arylamidase                    | w         | +         |
| Acid phosphatase                       | −         | +         |
| Naphthol-AS-BI-phosphohydrolase        | +         | −         |
| Acids production from                  |           |           |
| Glycerol                               | +         | −         |
| d-Adonitol I                           | +         | −         |
| d-Galactose                            | −         | +         |
| d-Fructose                             | +         | −         |
| Esculin                                | −         | +         |
| d-Sucrose                              | +         | −         |
| Xylitol                                | +         | −         |
| l-Fucose                               | +         | −         |
| Oxidation of carbon sources            |           |           |
| d-Cellobiose                           | −         | +         |
| Gentiobiose                            | −         | +         |
| d-Turanose                             | −         | +         |
| N-Acetyl-β-d-mannosamine               | −         | +         |
| 3-Methyl glucose                       | −         | +         |
| d-Glucose-6-PO<sub>4</sub>             | −         | +         |
| l-Aspartic acid                        | −         | +         |
| l-Glutamic acid                        | −         | +         |
| l-Histidine                            | −         | +         |
| α-Keto-butyric acid                    | −         | +         |
| Propionic acid                         | −         | +         |
| Polar lipids*                          | PME, PE, PG, PC, GL1, L-9 | PME, PE, PG, PC, GL1, L1-7, L10-13 |
| DNA G+C (mol%)                         | 69.8      | 67.9      |

Strains: 1, *L22<sup>T</sup>*; 2, *M. pamukkalensis JCM 30229<sup>T</sup>

All data were obtained from this study unless otherwise indicated+, positive; w, weakly positive; −, negative.

All strains are Gram-stain-negative, catalase and oxidase positive. The predominant respiratory quinone of all strains of the genus *Methylobrevis* is Q-10.

<sup>a</sup>Data from Poroshina et al. (2015)

<sup>PM</sup>E: phosphomonoester; *PE*: phosphatidylethanolamine; *PG*: phosphatidylglycerol; *PC*: phosphatidylcholine; *GL*: unidentified glycolipid; *L*: unidentified lipids.
The major fatty acid (> 10.0%) of strain L22T was summed feature 8 (C_{18:1ω7c}) (69.1%) same as the major fatty acid extracted from its related strain *M. pamukkalensis* JCM 30229T. The polar lipids of strain L22T comprised phosphomonoester (PME), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), one unidentified glycolipid (GL) and seven unidentified lipids (L). What deserved to be mentioned was that strain L22T and strain *M. pamukkalensis* JCM 30229T both possessed phosphononoester (PME), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylcholine (PC) as the major polar lipids (Fig. S3). Strain L22T contained unidentified lipids 1 to 9, while the strain *M. pamukkalensis* JCM 30229T did not have unidentified lipid 8 and unidentified lipids 1 to 9, but had unidentified lipid 10 to 13. Details of cellular fatty acids and polar lipids compositions are listed in Table 3. The major respiratory quinone of strain L22T was Q-10, which is in accordance to the description of the family Pleomorphomonadaceae (Hördt et al. 2020).

According to the phylogenetic analyses, differential chemotaxonomic data and other phenotypic properties, strain L22T was classified as a new member of the genus *Methylobrevis*.

**Table 3** Fatty acids compositions of strain L22T and related type strain

| Fatty acids | 1  | 2 |
|-------------|----|---|
| Branched    |    |   |
| Anteiso-C_{12:0} | 1.4 | Tr |
| Unsaturated |    |   |
| C_{16:0}11-methyl | 4.8 | Tr |
| Straight-chain |    |   |
| C_{12:0} | 1.4 | Tr |
| C_{18:0} | 3.9 | 2.4 |
| C_{16:0} | 4.4 | 6.3 |
| Hydroxylated |    |   |
| C_{16:0}3-OH | 1.6 | 1.1 |
| Unidentified |    |   |
| Summed Feature 2* | 2.7 | 2.6 |
| Summed Feature 3* | 3.9 | 5.7 |
| Summed Feature 8* | **69.1** | **71.2** |

Strains: 1, L22T; 2, *M. pamukkalensis* JCM 30229T

Data are from this study. Tr, traces (< 1.0%). Fatty acids amounting (< 1.0%) are not shown, dominant fatty acids (≥ 10.0%) are highlighted in bold.

Fatty acids 1 2

Strained feature 2 contained C_{12:0} and/or aldehyde

Summed feature 3 contained C_{16:0}7c and/or C_{16:1ω6c};

Summed feature 8 contained C_{18:1ω7c}

*Summed features are groups of two or three fatty acids that are treated together for evaluation in the MIDI system and include both peaks with discrete equivalent chain-lengths (ECLs) as well as those where the ECLs are not reported separately.
Declarations

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

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