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Original article

Polyphasic characterization of *Delftia acidovorans* ESM-1, a facultative methylotrophic bacterium isolated from rhizosphere of *Eruca sativa*

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ABSTRACT

In this study, one bacterial strain, ESM-1, was isolated from rhizosphere of *Eruca sativa*, growing in Al Hofouf, Saudia Arabia, after enrichment with methanol as a sole carbon and energy source in a batch culture. ESM-1 was characterized by a polyphasic approach. The strain was identified as *Delftia acidovorans* at similarity level of 99.9% of the 16S rRNA gene sequences. Results of the Biolog Gen III MicroPlate test system showed that strain ESM-1 reacted positively to 47 (50%) including the one-carbon compound formic acid, and partially positive to 6 (~6.4%) out of the 94 different the traits examined. The total cellular fatty acids composition of the strain ESM-1 was ($C_{16:1}\omega7c/C_{16:1}\omega6c$) and ($C_{16:0}$) and matched that of *Delftia acidovorans* at a similarity index of 0.9, providing a robustness to the ESM-1 identification. Furthermore, ESM-1 displayed a complex polar lipid profile consisting of phosphatidylethanolamine, phosphatidylglycerol, glycolipid, aminolipid, in addition to uncharacterized lipids. The DNA G+C content of the strain was 66.6 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain ESM1-1 was clearly clustered within the *Delftia* clade and constructed a monophyletic subcluster with *Delftia acidovorans* NBRC14950. The results addressed that ESM-1 is a facultative methylotrophic bacterium indigenous to Al Hofouf region and opens the door for potential biotechnological applications (e.g., bioremediation) of this strain, in future. Additionally, these findings assure that the total cellular fatty acid analysis and 16S rRNA gene are reliable tool for bacterial characterization and identification.

1. Introduction

Methylotrophy is the ability of certain microorganisms to metabolize one-carbon compounds, mainly methanol and/or methane, as their only carbon and energy source. In addition to bacteria, methylotrophic microorganisms include members of fungi and yeast. However, methanotrophic bacteria, a subset of methylotrophs, are mostly obligate methane utilizers. Certain species of *Methylocapsa, Methylocystis, Methylocella* could utilize two-carbon compounds (e.g., acetate and ethanol) in addition to methane, therefore they are called facultative methanotrophs (Dedysh and Dunfield, 2011). Substantial evidences have been confirmed that the methylotrophy is a more widespread phenomenon among diverse bacterial species than previously established (Chistoserdova et al., 2009). The reason for this could be attributed to the recent advances in the molecular and genetical tools, which facilitate in-depth studies of horizontal gene transfer and the isolation of bacteria from unexplored sites. Many bacterial species, which are taxonomically distant from the well-established methylobacteria, have shown the ability to utilize the C1 compounds. As an example, *Flavobacterium glycines* has been isolated from the rhizosphere of soybean (Madhaiyan et al., 2010). Additionally, novel methylotrophic bacterial species have proposed such as *Oharaeibacter diazotrophicus* from rice rhizosphere (Lv et al., 2017), *Methylobacillus caricis* from Carex sp. (Agafonova et al., 2017b), *Ancylobacter sonchi* from roots of *Sonchus arvensis* (Agafonova et al., 2017c), *Methylophaga muralis* from the Khilganta soda lake (Shmareva et al., 2018). Methylotrophs are ubiquitous in nature and inhabit diverse aquatic and terrestrial niches in addition to plant rhizosphere and phyllosphere. Methylobacteria exhibit multiple plant growth promoting activities such as symbiotic nitrogen fixation, production of phytohormonrs such as gibberellic

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acid, cytokines, and lowering ethylene level in plant roots by the activity of aminocyclopropane-1-carboxylate (ACC) deaminase (Agafonova et al., 2018). Furthermore, production of anti-phytopathogenic compounds (Yim et al., 2013) and phosphatases via which Phosphorous nutrients become available to the plants (Agafonova et al., 2013), are other approaches for plant promotion by methylobacteria. In addition, methylobacteria are potential active in carbon cycle and alleviating of global warming (Semrau, et al., 2018).

Members of the genus *Delftia* are aerobic, non-endospore forming, Gram-negative rods that inhabit diverse ecological niches. Taxonomically, this genus belongs *Comamonadaceae* family within the *Burkholderiales* order of the *Betaproteobacteria*. At the time of writing, six species are comprised within *Delftia* genus: *Delftia acidovorans*, isolated from soil (Wen et al., 1999), *Delftia tsuruhatensis*, isolated from activated sludge (Shigematsu et al., 2003); *Delftia lacustris*, isolated from freshwater (Jørgensen et al., 2009); *Delftia rhizosphaerae* isolated from the rhizosphere of *Cistus ladanifer* (Carro et al., 2017); *Delftia litopenaei* isolated from a freshwater shrimp culture pond (Chen et al., 2012); *Delftia deserti* isolated from a desert soil sample (Li et al., 2015). It has been reported that *Delftia* spp. have potential roles in bioremediation of organic and inorganic pollutants and production of industrially valuable compounds (Braña et al., 2016).

In Saudi Arabia, *Eruca sativa* L. (Rocket), a herb plant within the *Brassicacae* family, is used as an ingredient of green salad due to its high nutritional value and peculiar flavour (Lamy et al., 2008). *E. sativa* is locally known as Jarjeer and in addition to Najd and Hejaz, is cultivated in Eastern regions. Additionally, it has been reported that *E. sativa* has medicinal therapeutic activities such as protection of liver and inhibition of cancer and gastric ulcer (Alqasoumi, 2010). Rhizobacteria play a profound role in cleaning up soils from heavy metals via enhancing the uptake capability of *E. sativa* thereby reducing the environmental risk the phytoremediation roles. It has been reported that *Pseudomonas putida*, a plant growth promoting bacterium, enhances the bioaccumulation of cadmium and nickle (Kamran et al., 2016) by *E. sativa*, emphasizing its ecofriendly role.

Earlier studies in Al-Hofouf region were centered on methanotrophic bacteria using culture dependent and independent techniques (Al Malki and Khalifa 2017). Methylobacteria associated with *Eruca sativa* growing in Al-Hofouf, has not been previously studied. Therefore, the aim of the current study was to isolate and characterize methanol-degrading bacteria from rhizosphere *Eruca sativa* in Eastern region, Saudi Arabia. The obtained strain was characterized using polyphasic approach, which included phenotypic, genotypic and phylogenetic tools.

2. Material and methods

2.1. Collection of Eruca sativa plants and isolation of the strain ESM-1

Eruca sativa plants growing in Al Shehabiyah 25°21′49.0″N, 49°37′21.9″E, Al Hofuf Eastern region, Saudi Arabia, were carefully uprooted and collected along with rhizosphere soils in sterilized plastic bags in March 1st, 2017. Exactly 0.5 g of rhizosphere was enriched with (0.5%v/v) methanol as the only carbon source in a 250 ml conical flask containing 50 ml of the Nitrate Mineral Salts (NMS) liquid medium (Bowman, 2006). Flasks were incubated at 30 °C in a shaking-incubator at 150 rpm m^{-1} for 7 days. Then, 100 μl aliquots from the methanol-enriched media were streaked into NMS agar plates containing 0.5% methanol and were incubated at 30 °C for 5 days. Discrete colonies were further streaked into fresh NMS agar. Preservation of isolates was carried out by streaking on NMS plates every 2–3 weeks, in a regular basis.

2.2. Morphological characteristics of the strain ESM-1

The 3-days old colonies of the ESM-1, growing on NMS agar plates containing 0.5% methanol and incubated at 30 °C, were morphologically characterized. Furthermore, the cells were stained using Gram-staining reaction.

2.3. Scanning electron microscopic investigation of the strain ESM-1

Discrete pure colonies of the strain ESM-1 was investigated under scanning electron microscopy (Joel) as previously described (Khalifa and Bekhit, 2017).

2.4. Characterizations of the strain ESM-1 using Biolog Gen III microtest system

In order to determine the phenotypic characteristics of the strain ESM-1 m the Biolog Gen III microtest system (Biolog, USA) was applied typically as indicated by the manufacturer. After 24 h of incubation at 30 °C, the results were obtained. Negative and positive controls were contained in two wells in this system where colourless and purple appearance were reported, respectively.

2.5. Identification of the strain ESM-1 using the 16S rRNA gene sequencing

In order to identify the strain ESM-1 accurately, 16S rRNA gene sequencing was applied. Extraction of genomic DNA, amplification of the target gene with the universal primers, PCR conditions and sequencing of the purified amplicon were performed as described earlier (Khalifa and Bekhit, 2017).

2.6. Analysis of the cellular fatty acids of the strain ESM-1

The strain ESM-1 was grown on Tryptic Soy Broth Agar (TSBA, Himedia) at 28 °C for 48 h. Cellular fatty acid analysis was performed as described by Sasser (1990) according to MIDI protocol by gas chromatography with flame ionization detector (GC-FID). Microbial Identification Software (MIDI Sherlock aerobe method and TSBA library version Aerobic Bacteria Library (TSBA6/RTSBA, 6v 6.10) Newark, DE, USA), was used. Reference means peaks of that particular species in the MIDI database.

2.7. Determination of polar lipids for the strain ESM-1

The cultures were harvested at log phase and the pellet was freeze-dried which was used for polar lipid extraction with methanol/chloroform 0.3% sodium chloride (2:1:0.8, by vol.) as described by Bligh and Dryer (1959) considering the modifications of Card (1973). Lipids were separated using silica gel TLC (Kieselgel 60F254, Merck) by two- dimensional chromatography using chloroform-methanol-water (65:25:15.4, by vol.) in the second dimension (Tindall, 1990). The dried plates were subjected to spraying with 5% ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates), Dragendroff (quaternary nitrogen) or alpha naphthol (specific for sugars).

2.8. Construction of a phylogenetic tree for the strain ESM-1

The 16S rRNA gene sequences of the strain ESM-1 along with other sequences of closely related strains were used to construct a neighbour-joining tree based on the Tamura-Nei model (Tamura and Nei, 1993), including all codon positions, to reveal the phylogenetic relationships. Exactly, 1000 bootstrap replicates were applied for determining the branch support. Alignments of sequences and phylogenetic analyses were carried out using the

freely available software MEGA 7 (Kumar et al., 2016). The 16S rRNA gene sequences of the ESM-1 has been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and the accession number (MG847185).

2.9. Determination of the GC content of the strain ESM-1

Thermal denaturation was performed with 1 μg DNA in each well along with a fluorescent dye SYBR Green I (Invitrogen) at a final dilution 1:100,000. Thermal conditions comprised in a ramp from 25 °C to 100 °C at a 1 °C min^{-1} were achieved by using StepOnePlus Real Time PCR system (Applied Biosystems) fitted with 96 well thermal cycling block in 96 well plate. Fluorescence reading was considered at each step during the ramp. Tm based G+C by fluorimetric method with normalized reporter plot settings was done in triplicates as described by Gonzalez and Saiz-Jimenez (2002).

3. Results and discussion

A bacterial strain designated ESM-1 was isolated from rhizosphere from *Eruca sativa* growing in Al Hofouf, on MNS medium containing methanol as a sole source of carbon and energy. The external features of the colonies formed by the strains were presented in Table 1. ESM-1 formed a circular, smooth and cream colour with entire edge. Cells were rod-shaped with no endospores (Fig. 1).

3.1. Characterizations of the strain ESM-1 using Biolog Gen III microtest system

The results based on the Biolog Gen III MicroPlate test system is presented in Table 1. Strain ESM-1 exhibited the ability to react positively to 47 (50%) and partially positive to 6 (~6.4%) out of the 94 different physiological and biochemical traits examined (Table 2). Nonetheless, ESM-1 was unable to grow on 42 (~43.6%) testers. Strain ESM-1 grew on a wide range of sugars (e.g., α-D-glucose, D-fructose, sucrose), polyvalent alcohols (e.g., D-Mannitol), hexose-PO_4 (e.g., D-Fructose-6-PO_4), carboxylic acids (e.g., D-Malic Acid) and heteropolysaccharide (e.g., pectin) (Table 2). Additionally, certain amino acids (e.g., L-Arginine) and proteins (e.g., gelatin) were metabolized by the strain ESM-1. Growth on lithium chloride and at pH 6 was also reported. However, ESM-1 did not grow on many substrate tested such as D-Raffinose, D-Glucose-6-PO_4, D-Sorbitol, Citric Acid, L-Alanine. No growth was observed on any of the NaCl concentration (e.g., 1%), antibiotics (Vancomycin) and dyes (e.g., Tetrazolium Blue) tested at 1, 4 and 8% NaCl (Table 2). Weak growth was noticed in 6 (~6.4%) tests (Table 2). Such borderline growth was on p-Hydroxy- Phenylacetic Acid, α-D-Lactose, Glycyl-L-Proline, Inosine, Aztreonam and Tetrazolium Violet (Table 2). The strain ESM-1 consumed many of the chemical compounds as carbon and nitrogen sources and coped with certain stressors investigated using the Biolog Gen III MicroPlate test system, highlighting diverse metabolic pathways of this bacterium. These results were in general agreement with those

Table 1
Morphological and genetical characteristics of the strain ESM-1.

Characteristic	Result
Colony morphology	Circular and smooth colony with entire edge
Pigmentation	Cream
Gram staining	Negative
Cells	Rod-shaped
DNA C+G content	66.6 ± 0.3 mol%.
Identity percentage 16S rRNA gene sequence	99.9% to *Delftia acidovorans* NBRC14950
NCBI 16S rRNA gene sequence accession No.	MG847185

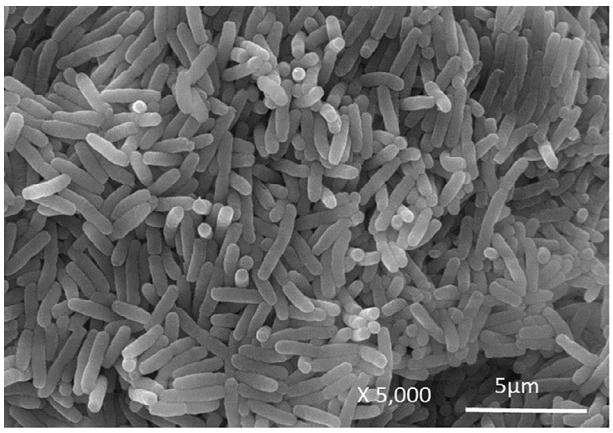

Fig. 1. A scanning electron micrograph shows the cell shape and arrangement of the strain ESM-1. Scale bar and magnification and are shown at the bottom of the image.

Table 2
Characterization of the strain ESM-1 based on the Biolog Gen III microplate.

Positive reaction with the following substrate/test		
Gelatin	L-Lactic Acid	L-Fucose
Pectin	β-Hydroxy-D,L-Butyric Acid	D-Fructose-6-PO4
Tween 40	D-Cellobiose	L-Histidine
Dextrin	D-Salicin	D-Malic Acid
D-Mannitol	3-Methyl Glucose	N-Acetyl-D-Galactosamine
Methyl Pyruvate	Glycerol	L-Rhamnose
γ-Amino-Butryric Acid	L-Aspartic Acid	L-Pyroglutamic Acid
D-Fructose	D-Glucuronic Acid	L-Malic Acid
D-Arabitol	α-Keto-Butyric Acid	Acetic Acid
L-Galactonic Acid Lactone	Gentiobiose	D-Saccharic Acid
D-Trehalose	N-Acetyl-D-Glucosamine	Formic Acid
β-Methyl-D-Glucoside	Glucuronamide	Positive Control
D-Galactose	α-Keto-Glutaric Acid	1% Sodium Lactate
myo-Inositol	Acetoacetic Acid	pH 6
L-Arginine	Sucrose	Lithium Chloride
D-Gluconic Acid	N-Acetyl-b-D-Mannosamine	
Weak positive reaction with the following substrate/test		
p-Hydroxy-Phenylacetic Acid	Glycyl-L-Proline	Aztreonam
α-D-Lactose	Inosine	Tetrazolium Violet
Negative reaction with the following substrate/test		
D-Raffinose	Mucic Acid	Nalidixic Acid
α-D-Glucose	Propionic Acid	Fusidic Acid
D-Sorbitol	D-Turanose	Rifamycin SV
D-Mannose	D-Aspartic Acid	Guanidine HCl
D-Galacturonic Acid	Quinic Acid	Sodium Butyrate
D-Maltose	Stachyose	pH 5
D-Melibiose	N-Acetyl Neuraminic Acid	8% NaCl
L-Alanine	D-Serine	D-Serine
D-Lactic Acid Methyl Ester	L-Serine	Minocycline
α-Hydroxy- Butyric Acid	Bromo-Succinic Acid	Niaproof 4
Citric Acid	1% NaCl	Tetrazolium Blue
D-Fucose	Troleandomycin	Potassium Tellurite
D-Glucose-6-PO4	Lincomycin	Sodium Bromate
L-Glutamic Acid	Vancomycin	

determined with *Delftia* spp. (Agafonova et al., 2017a, Wen et al., 1999).

It is evident that the Biolog GEN III Microsystem is efficiently applied for assessing the biochemical and physiological characteristics of the novel proposed *Delftia* species such as *Delftia deserti* (Li et al., 2015) and other taxa such as *Limoniibacter endophyticus* (Li et al., 2018).

The ability of the strain ESM-1 to metabolize chitin is attributed to chitinase enzyme. Similar results have been reported for *Delftia* sp., *Bacillus subtilis* and *B. cereus*. Chitinolytic activities could destroy the integrity of the cell wall of fungal phytopathogens, therefore inhibiting their growth (Jørgensen et al., 2009). In addition to methanol, ESM-1 grew on formic acid, a C1 compound, as a sole source of carbon and energy, highlighting the methylotrophic nature of this bacterium. These findings are in accordance with that reported recently by Agafonova et al. (2017a) who provided the first comprehensive description of a facultative methylotrophic strain, Lp-1, within the genus *Delftia*. Lp-1 was isolated from root-nodules of *Lupinus polyphyllus* and exhibited a substantially high level of the 16S rRNA gene sequence similarity (99.9%) with *D. lacustris* 332^T. With the exception of *D. tsuruhatensis* BM90 (Juarez-Jimenez et al., 2010) and *Delftia* Lp-1 (Agafonova et al., 2017a), the capabilities of the *Delftia* spp. to metabolize C1 compounds have not been reported. The results confirmed the presence of another methylotrophic strain that inhabit rhizosphere of *E. sativa*. Additionally, *Flavobacterium glycines* (Madhaiyan et al., 2010), and certain members within *Actinobacteria*, *Sphingobacteriia* and *Proteobacteria* (del Rocío et al., 2017) were documented as facultative methylotrophs, confirming the widespread occurrence of methylotrophy among taxonomically different species. Horizontal gene transfer of the genetic elements responsible for methylotrophy to non-methylotrophic strains could explain this phenomenon (Chistoserdova et al., 2009, Chistoserdova, 2015). In the same line of that, novel methanol dehydrogeneases and low-affinity monooxygenases have been revealed (Taubert et al., 2015).

Mining the whole genome sequences of *Delftia acidovorans* RAY209, which has been recently released, (Perry et al., 2017), revealed the presence of the pyrroloquinoline quinone (PQQ) dependent methanol dehydrogenase, an enzyme responsible for methanol oxidation. PQQ is active catalytic center of this enzyme. Therefore, ESM-1 is likely to oxidize methanol using PQQ-dependent methanol dehydrogenase. However, experimental estimation of methanol oxidation via measuring the enzymatic activities has to be done in future work.

3.2. Identification of the strain ESM-1 using the 16S rRNA gene sequencing

One of the most convenient and accurate method for prokaryotic classification and identification is the comparing of the 16S rRNA gene sequence of a particular isolate against sequences of all recognized reference bacterial strains with validly published names using a well-curated databases. EzTaxon, a well-curated database, was selected for ESM1- identification (Kim and Chun, 2014). Strain ESM-1 (accession number: MG847185) constructed a monophyletic subcluster with *Delftia acidovorans* NBRC14950 (Fig. 2) ESM-1 exhibited 16S rRNA gene sequence similarity of 99.9% to *Delftia acidovorans* 2167 (Table 1); 99.43% *Delftia lacustris* LMG 24775; 99.43% *Delftia tsuruhatensis* NBRC 16741; 99.3% *Delftia litopenaei* wsw-7; 98.1% *Delftia rhizosphaerae* RA6; 96.4%.

3.3. Phylogenetic analyses

It is well established that the evolutionary history and relationships among bacterial species could be precisely inferred from sequences of 16S ribosomal RNA gene (Woese, 1987) and/or those of other housekeeping genes. Phylogenetic analyses based on 16S rRNA gene sequences showed that the strain ESM1-1 was clearly clustered within the *Delftia* clade (Fig. 2) as constructed with the Neighbor-Joining method (Saitou and Nei, 1987) using the MEGA7

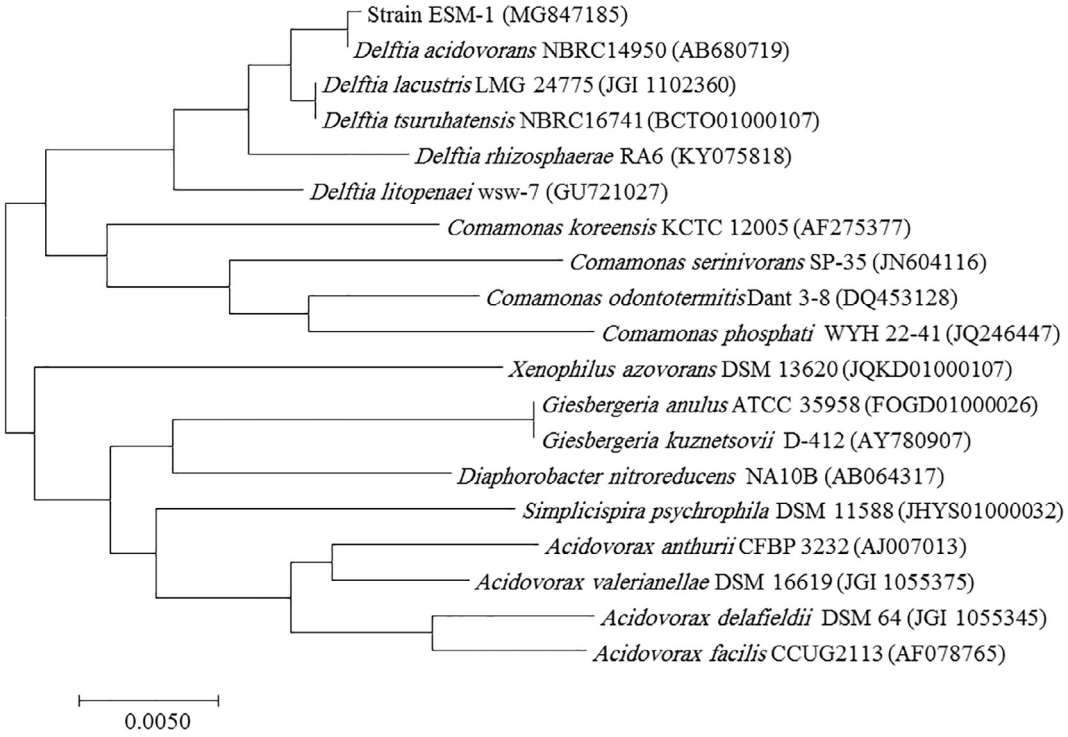

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *D. acidovorans* ESM-1 and related taxa in the order *Burkholderiales* of the class *Betaproteobacteria*. Bar, 0.0050 substitutions per nucleotide position.

software (Kumar et al., 2016). Similar overall topology of phylogenetic tree was obtained using the maximum-parsimony (Nei and Kumar, 2000) and maximum likelihood methods (Tamura and Nei, 1993), providing a reliable position of the strain ESM-1 within phylogenetic trees (Data mot shown). Nonetheless, based on the phenotypic and phylogenetic characterization *Comamonas acidovorans* or *Pseudomonas acidovorans* was reclassified as *D. acidovorans* (Wen et al., 1999).

3.4. The total cellular fatty acids composition

The total cellular fatty acids composition of the strain ESM-1, as determined by the MIDI system is shown in Table 3. Generally, the saturated and unsaturated fatty acids were detected in the strain ESM-1. The major fatty acids were those from summed feature 3 (hexadecenoic acid) ($C^{\omega7c}_{16:1}/C^{\omega6c}_{16:1}$), (hexadecanoic acid) $C_{16:0}$ and (octadecenoic acid) $C^{w7c}_{18:1}$ with proportions of 40.73%, 31.13% and 19.18%, respectively (Table 3). Collectively, those fatty acids represent greater than 91% of the total cellular fatty acids of the strain ESM-1. The rest of the fatty acids such as $C^{3\text{-OH}}_{8:0}$, $C^{3\text{-OH}}_{10:0}$, $C_{12:0}$, $C_{14:0}$ were reported as minor ones as they represent less than 9% of the total cellular fatty acids. These data are typically in accordance with those reported for the type strain of the *Delftia acidovorans* (Wen et al., 1999), providing robustness for identification of this strain. The similarity index 'Sim Index' was 0.902, which was matched *Delftia acidovorans* as indicated by the MIDI report (S1-S3). It is well known that the Sim index value reflects the similarity of the fatty acids profile in the library of MIDI system with that of the sample analyzed. Therefore, the strain ESM-1 is identified as *Delftia acidovorans* based on the total fatty acid profile. This finding clearly provides a robustness of the ESM-1 identification based of the comparative sequencing of the 16S rRNA gene. Additionally, this observation assures that the total cellular fatty acid analysis is a reliable tool for bacterial characterization and identification. It has been reported that a comprehensive lipid analysis is an efficient tool for bacterial classification at the species level.

3.5. Polar lipid profile

Strain ESM-1 displayed a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), glycolipid (GL), aminolipid (AL), an uncharacterized phospholipid (UPL) and an uncharacterized lipid (UL) (Fig. 3). Compared with its closest relatives, *D. acidovorans* NBRC14950^T, *D. deserti* YIM y792^T, *D. tsuruhatensis* NBRC 16741^T and *D. lacustris* DSM 21246^T, strain EMS-1 showed a very similar polar lipid profile, and they all had PE, PG, GL, UPL and UL (Chen et al., 2012). Unlike the reference strains, DPG was absent in the strain ESM-1. Additionally, PL3 and PL4 were not detected in strain ESM-1. PL4 was only detected

Table 3
Cellular fatty acid composition of the strain ESM-1.

Fatty acids	Percentage*
$C^{3\text{-OH}}_{8:0}$	0.37
$C^{3\text{-OH}}_{10:0}$	2.95
$C_{12:0}$	2.56
$C_{14:0}$	0.84
$C^{\omega7c}_{16:1}/C^{\omega6c}_{16:1}$ **	40.73
$C_{16:0}$	31.13
$C_{17:0}$ cyclo	1.57
$C_{17:0}$	0.26
$C^{w7c}_{18:1}$	19.18
$C_{18:0}$	0.43

* Values are percentages of whole cellular fatty acids.
** $C^{\omega7c}_{16:1}/C^{\omega6c}_{16:1}$ were reported as 'Summed feature' based on the report using the MIDI system.

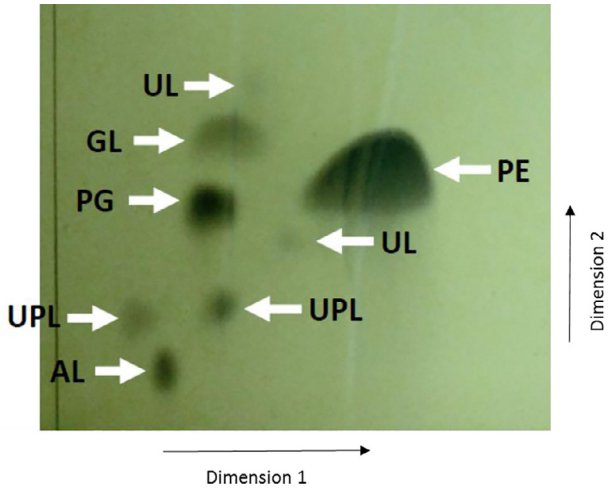

Fig. 3. Polar lipid analysis of the strain ESM-1, phosphatidylethanolamine (PE); phosphatidylglycerol (PG); glycolipid (GL); aminolipid (AL); an uncharacterized phospholipid (UPL) and an uncharacterized lipid (UL).

in *D. litopenaei* wsw-7^T and PL3 was present in both *D. acidovorans* ATCC15668^T and *D. litopenaei* wsw-7^T (Li et al., 2015). These findings highlighted that minor differences in the polar lipid profiles exist among different species within the same genus although they commonly have very similar profiles.

3.6. Determination of the DNA G+C content of the strain ESM-1

It is well established that the genomic DNA G+C content, known as the percentage of guanines and cytosines within the total number of nucleotides of a particular genome, varies among species and genera. The DNA C+G content is potential feature that is commonly estimated for description of bacterial taxa (Mesbah et al., 2011). The DNA C+G content of the strain ESM-1 as determined by the thermal denaturation method was 66.6 ± 0.3 mol% (Table 1). This finding is identical with that reported with *Delftia acidovorans* RAY209 (Perry et al., 2017) and slightly lower than that (67 mol%.) reported with *Delftia acidovorans* ATCC 15668^T (Wen et al., 1999). It has been suggested that an accurate estimation of the G+C content could be done via whole genome sequences owing to the rapid advances in sequencing technologies and the marked drop in cost (Meier-Kolthoff et al., 2014).

Collectively, one facultative methylotrophic bacterium, ESM-1, was obtained from the rhizosphere of *E. sativa* growing in Al Hofouf region Saudi Arabia. Polyphasic characterization identified the strain as *Delftia acidovorans* confirming that methylotrophic capability is widespread in diverse bacterial taxa and not restricted to a particular group of bacteria as previously suggested. The metabolic versatility of the strain ESM-1 could be the base for promising industrial and agricultural applications, in future.

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