Enterobacter muelleri sp. nov., isolated from the rhizosphere of *Zea mays*

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A beige-pigmented, oxidase-negative bacterial strain (JM-458^T), isolated from a rhizosphere sample, was studied using a polyphasic taxonomic approach. Cells of the isolate were rod-shaped and stained Gram-negative. A comparison of the 16S rRNA gene sequence of strain JM-458^T with sequences of the type strains of closely related species of the genus *Enterobacter* showed that it shared highest sequence similarity with *Enterobacter mori* (98.7 %), *Enterobacter hormaechei* (98.3 %), *Enterobacter cloacae* subsp. *dissolvens*, *Enterobacter ludwigii* and *Enterobacter asburiae* (all 98.2 %). 16S rRNA gene sequence similarities to all other *Enterobacter* species were below 98 %. Multilocus sequence analysis based on concatenated partial *rpoB*, *gyrB, infB* and *atpD* gene sequences showed a clear distinction of strain JM-458^T from its closest related type strains. The fatty acid profile of the strain consisted of $C_{16:0}$, $C_{17:0}$ cyclo, iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ and $C_{18:1}\omega7c$ as major components. DNA–DNA hybridizations between strain JM-458^T and the type strains of *E. mori*, *E. hormaechei* and *E. ludwigii* resulted in relatedness values of 29 % (reciprocal 25 %), 24 % (reciprocal 43 %) and 16 % (reciprocal 17 %), respectively. DNA–DNA hybridization results together with multilocus sequence analysis results and differential biochemical and chemotaxonomic properties showed that strain JM-458^T represents a novel species of the genus *Enterobacter*, for which the name *Enterobacter muelleri* sp. nov. is proposed. The type strain is JM-458^T (=DSM 29346^T=CIP 110826^T=LMG 28480^T=CCM 8546^T).

The genus *Enterobacter* is a polyphyletic genus within the *Enterobacteriaceae*. Several species of the genus have been reclassified in recent years based on phylogenetic reanalysis, including multilocus sequence analysis (MLSA), into novel genera (Brady *et al.*, 2013; Oren & Garrity, 2013, 2014a; Stephan *et al.*, 2014). The overall phylogeny of the genus remains unresolved (Brady *et al.*, 2013; Gu *et al.*, 2014; Stephan *et al.*, 2014). There remain several species from distinct branches within the *Enterobacteriaceae* that indicate the need for further reclassifications of this polyphyletic genus. At the time of writing, recognized species/subspecies in the genus *Enterobacter* (http://www.bacterio.cict.fr/e/enterobacter.html) include *Enterobacter cloacae* subsp. *cloacae* (type species) and *Enterobacter cloacae* subsp. *dissolvens*, *Enterobacter mori*, *Enterobacter hormaechei* and *Enterobacter ludwigii*, and *Enterobacter soli* and *Enterobacter kobei*, which form at least three distinct clades based on 16S rRNA gene sequence phylogeny but cluster together in the MLSA-based phylogenetic tree (Brady *et al.*, 2013). Recently proposed species include *Enterobacter siamensis* (Khunthongpan *et al.*, 2013; Oren & Garrity, 2014a) and *Enterobacter xiangfangensis* (Gu *et al.*, 2014), which cluster together with other *Enterobacter* species, as well as *Enterobacter massiliensis* (Lagier *et al.*, 2013; Oren & Garrity, 2014b), which form a separate branch in the 16S rRNA gene sequence-based phylogeny (see below).

Enterobacter species have been reported as both notorious plant pathogens and human opportunistic pathogens (Chow *et al.*, 1991; Chung *et al.*, 1993; Nishijima *et al.*, 2007), and also as important engineering and plant growth-promoting bacteria (French *et al.*, 1998; Nie *et al.*, 2002). Some *Enterobacter* strains may play important roles in plant–microbe interactions and hence in biocontrol mechanisms (Chernin *et al.*, 1996).

A beige-pigmented strain (JM-458^T) was isolated on nutrient-agar (NA; Oxoid) from a rhizosphere sample. It displayed the typical colony morphological properties of *Enterobacteriaceae*. The source of the organism was the rhizosphere of 1-week-old corn (*Zea mays*, cultivar 'Sweet Belle') grown at the Plant Breeding unit facility at the E.V. Smith Research Center in Tallassee (Elmore county), AL, USA.

Abbreviations: LTP, 'All-Species Living Tree' Project; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *rpoB*, *gyrB*, *infB* and *atpD* gene sequences of strain JM-458^T are KP345900, KP345902, KP345909, KP345905 and KP345913, respectively.

One supplementary table and four supplementary figures are available with the online Supplementary Material.

Strain JM-458^T was maintained and subcultivated on NA at 30 °C for 48 h and subsequently used for 16S rRNA gene sequence analysis, MLSA based on concatenated partial *rpoB*, *gyrB*, *infB* and *atpD* gene sequences, and analyses of fatty acid methyl ester composition of whole-cell hydrolysates, further phenotypic features and DNA–DNA relatedness to those *Enterobacter* species most closely related on the basis of 16S rRNA gene sequence similarities. Reference strains for physiological and biochemical tests and fatty acid analysis (*E. mori* LMG 25706^T, *E. hormaechei* EN 314^T and *E. ludwigii* EN-119^T) and further reference strains (DNA–DNA hybridization analysis) were grown under the same conditions as the novel strain.

Cultural and morphological characteristics were determined from cultures grown on NA at 28 °C. The Gram reaction was tested on the basis of a modified method of Gerhardt *et al.* (1994). Cell size and motility were analysed under a light microscope at 1000-fold magnification (Axiophot 2, Zeiss) on cells grown for 3 days in nutrient broth (Oxoid) at 30 °C. Oxidase activity was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Catalase activity was tested by gas formation after saturation with H_2O_2 of fresh biomass grown for 48 h on NA. Growth was investigated at different temperatures (4, 8, 10, 30, 37, 45 and 50 °C) on NA. NaCl tolerance was evaluated in trypticase soy broth (TSB; Oxoid) supplemented with 0.5–10.0 % (w/v) NaCl.

Strain JM-458^T showed a Gram-negative staining behaviour and formed visible (diameter about 2 mm) beige colonies within 48 h at 30 °C. It was negative for oxidase and catalase activity. Cells were motile, non-spore-forming rods (approximately 1 μm wide and 2 μm long). Strain JM-458^T grew well on NA, brain heart infusion agar, R2A agar, TSA and MacConkey agar (Oxoid). No growth was observed below 8 °C or above 45 °C. Strain JM-458^T also grew with 0–6 % (w/v) NaCl, weakly in the presence of 8 and 9 % NaCl but not with 10 % NaCl or in TSB adjusted to pH 4.5–11.5.

Detailed physiological characterization and biochemical tests were performed to assess the carbon source utilization pattern and hydrolysis of chromogenic substrates as described by Kämpfer *et al.* (1991). Additional biochemical tests were performed: production of hydrogen sulphide, indole reaction with Ehrlich's and Kovacs' reagents, and activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, β-galactosidase (ONPG) and urease on the basis of the methods described by Kämpfer (1990). The strain utilized many carbon sources, similar to all species of the genus *Enterobacter*, and was able to hydrolyse many chromogenic substrates. The biochemical/physiological data are given in Table 1 and in the species description and allowed clear differentiation from its most closely related *Enterobacter* species.

The 16S rRNA gene of strain JM-458^T was PCR-amplified and sequenced with universal 16S rRNA gene targeting primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′; Lane, 1991). First phylogenetic identification including sequence similarity

Table 1. Phenotypic characteristics that differentiate strain JM-458^T from related species of the genus *Enterobacter*

Taxa: 1, JM-458^T; 2, *E. mori* R18-2^T and R3-3; 3, *E. asburiae* ATCC 35953^T; 4, *E. asburiae* (Hoffmann *et al.*, 2005); 5, *E. cancerogenus* LMG 2693^T; 6, *E. cancerogenus* (Stephan *et al.*, 2007); 7, *E. cloacae* subsp. *cloacae* ATCC 130477^T; 8, *E. cloacae* subsp. *cloacae* (Kämpfer *et al.*, 2005); 9, *E. cloacae* subsp. *dissolvens* LMG 2683^T; 10, *E. cloacae* subsp. *dissolvens* (Hoffmann *et al.*, 2005); 11, *E. hormaechei* (Kämpfer *et al.*, 2005); 12, *E. ludwigii* (Kämpfer *et al.*, 2005). Except for taxon 1, data are from Zhu *et al.* (2011), and were confirmed for type strains 2–5 in this study. When more than one strain was analysed, the percentage of strains giving a positive result is scored as: −, 0–10 % positive; (−), 10–20 % positive; V, 20–80 % positive; (+), 80–90 % positive; +, 90–100 % positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Methyl red test	+	−	+	+	−	−	−	−	−	−	−	−
Arginine dihydrolase	+	+	V	V	+	+	+	+	+	+	V	+
Motility	+	+	V	(−)	+	+	+	+	(−)	−	V	+
Aesculin hydrolysis	+	+	+	+	(+)	+	(+)	V	+	+	−	(−)
Lysine decarboxylase	−	+	−	−	−	−	−	−	−	−	−	−
Carbon source utilization:												
Sucrose	+	+	+	+	−	−	+	+	+	+	+	+
Melibiose	−	+	−	−	−	−	(+)	+	+	+	(−)	+
D-Arabitol	−	+	−	−	(−)	−	V	(−)	−	−	(−)	−
D-Sorbitol	+	+	+	(+)	−	−	+	+	+	+	(−)	+
L-Fucose	ND	V	−	−	+	+	−	−	V	V	+	V
Putrescine	−	+	(−)	(−)	+	+	+	+	+	+	−	−
α-L-Rhamnose	−	+	(+)	(+)	+	+	+	+	(+)	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+	+
Mucate	ND	+	V	V	+	+	V	V	+	+	+	+
1-*O*-Methyl α-galactopyranoside	ND	V	(+)	(+)	_	_	+	+	+	+	_	+

analysis was performed in the EzTaxon type strain 16S rRNA gene sequence database (Kim *et al.*, 2012). Detailed phylogenetic analyses based on nearly full-length 16S rRNA gene sequences were performed in ARB release 5.2 (Ludwig *et al.*, 2004) using the 'All-Species Living Tree' Project (LTP; Yarza *et al.*, 2008) database release LTPs119 (November 2014). Sequences not included in the LTP database were used after alignment with SINA (v. 1.2.11; Pruesse *et al.*, 2012). The final alignment including all sequences used for tree reconstruction was checked manually based on secondary structure information. Pairwise sequence similarities were calculated in ARB using the ARB neighbour-joining tool without the application of an evolutionary model. Phylogenetic trees were reconstructed with the maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis and with PhyML using the HKY85 model and the maximum-parsimony method using DNAPARS v 3.6 (Felsenstein, 2005) based on 100 resamplings (bootstrap analysis; Felsenstein, 1985). All trees were based on 16S rRNA gene sequences between gene termini 107 and 1378 (according to the *Escherichia coli* numbering scheme; Brosius *et al.*, 1978).

The sequenced 16S rRNA gene fragment of strain JM-458^T was a continuous stretch of 1422 unambiguous nucleotides (termini 48–1467; *E. coli* numbering). Highest pairwise sequence similarities to related *Enterobacter* species were 98.7 % to *E. mori* R18-2^T, 98.3 % to *E. hormaechei* CIP 103441^T, and 98.2 % to *E. cloacae* subsp. *dissolvens* LMG 2683^T, *E. ludwigii* EN-119^T and *Enterobacter asburiae* JCM 6051^T. Sequence similarities to the type strains of all other *Enterobacter* species were below 98 %. Additionally, strain JM-458^T showed greater than 98 % 16S rRNA gene sequence similarity to *Leclercia adecarboxylata* CIP 82.92^T (98.2 %) and *Cedecea davisae* DSM 4568^T. In phylogenetic trees reconstructed with the different treeing methods, strain JM−458^T clustered with *E. mori* R18-2^T, *L. adecarboxylata* CIP 82.92^T, *E. ludwigii* EN-119^T, *E. cancerogenus* LMG 2693^T, *E. asburiae* JCM 6051^T, *E. hormaechei* CIP 103441^T and *E. xiangfangensis* 10-17^T (Fig. 1) and dependent on the applied treeing method also with *E. cloacae* subsp. *dissolvens* LMG 2683^T, *E. cloacae* subsp. *cloacae* ATCC 13047^T and *E. siamensis* C2361^T, but not with *E. massiliensis* JC163^T which formed a distinct branch separated from the other *Enterobacter* species (Fig. 1). Clustering among the closely related *Enterobacter* species was strongly affected by the treeing method used, caused by the high gene sequence similarity among the species. The clustering was not supported by high bootstrap values in any of the applied treeing methods, illustrating the problem of resolution for *Enterobacter* species based on 16S rRNA gene sequence phylogenetic identification.

For a more detailed phylogenetic analysis, MLSA was performed based on concatenated sequences of partial *rpoB*, *gyrB*, *infB* and *atpD* nucleotide sequences (635, 739, 607 and 642 nt, respectively) and amino acid sequences (211, 246, 202 and 214 aa, respectively). PCR amplification and sequencing were performed as described by Brady *et al.* (2008). Manual sequence correction and phylogenetic analysis were performed in MEGA5 (Tamura *et al.*, 2011) using reference sequences from GenBank (http://www.ncbi.nlm.nih.gov/). All sequences included here are listed in Table S1 (available in the online Supplementary Material). The full-length gene sequences of genome-sequenced reference strains (Table S1) were used to determine the correct ORF of partial sequences. Nucleotide alignments were performed based on respective amino acid alignments. Maximum-likelihood trees were reconstructed using the general time resolved (GTR) model by using a discrete gamma-distribution (+ G) with five rate categories and by assuming that a certain fraction of sides are evolutionary invariable (+ I) (for nucleotide sequences) and the Jones–Thornton–Taylor model (JTT; Jones *et al.*, 1992) + G + I (for amino acid sequences). Best fitting substitution models were determined with the Bayesian information criterion (Schwarz, 1978) using the maximum-likelihood model test implemented in MEGA 5. All phylogenetic trees were based on 100 replications (bootstraps). In contrast to the 16S rRNA gene-based analysis, strain JM-458^T clustered with *E. cloacae* subsp. *cloacae* LMG 2783^T and *E. cloacae* subsp. *dissolvens* LMG 2683^T in maximum-likelihood trees reconstructed based on concatenated *rpoB*, *gyrB*, *infB* and *atpD* nucleotide and amino acid sequences (Fig. 2). The clustering of strain JM-458^T in phylogenetic trees reconstructed based on individual partial nucleotide gene sequences showed slight differences amongst each other, especially with respect to the clustering to closest related taxa (Figs S1–S4). Strain JM-458^T clustered closest either to the type strains of *E. mori* and *E. asburiae* (*rpoB*, *gyrB*, *atpD*) or to the type strains of the two subspecies of *E. cloacae* (*infB*). A similar clustering was obtained at the level of amino acids, but several *Enterobacteriaceae* species including those of the genus *Enterobacter* and strain JM-458^T could not be differentiated based on partial RpoB and RecA sequences (Figs S1 and S4). Sequence similarities of strain JM-458^T were investigated in more detail for concatenated nucleotide and amino acid sequences. At the nucleotide level the clustering was supported by a high bootstrap value. Based on nucleotide sequences, strain JM-458^T showed similarities of 96.1–96.4 % to the two subspecies of *E. cloacae* but less than 96 % to all other investigated *Enterobacter* species. At the amino acid level, strain JM-458^T shared 99.1 % sequence similarity to the two subspecies of *E. cloacae* but less than 98.5 % to the most closely related *Enterobacter* based on 16S rRNA gene sequence analysis. Strain JM-458^T showed no direct clustering to *L. adecarboxylata*, either at the nucleotide or at the amino acid sequence level, although they did cluster together in the 16S rRNA gene sequence-based trees.

DNA–DNA hybridization experiments were performed between strain JM-458^T and the type strains of the three most closely related *Enterobacter* species (>98.3 % 16S rRNA gene sequence similarity) and the type species of the genus according to the method of Ziemke *et al.*

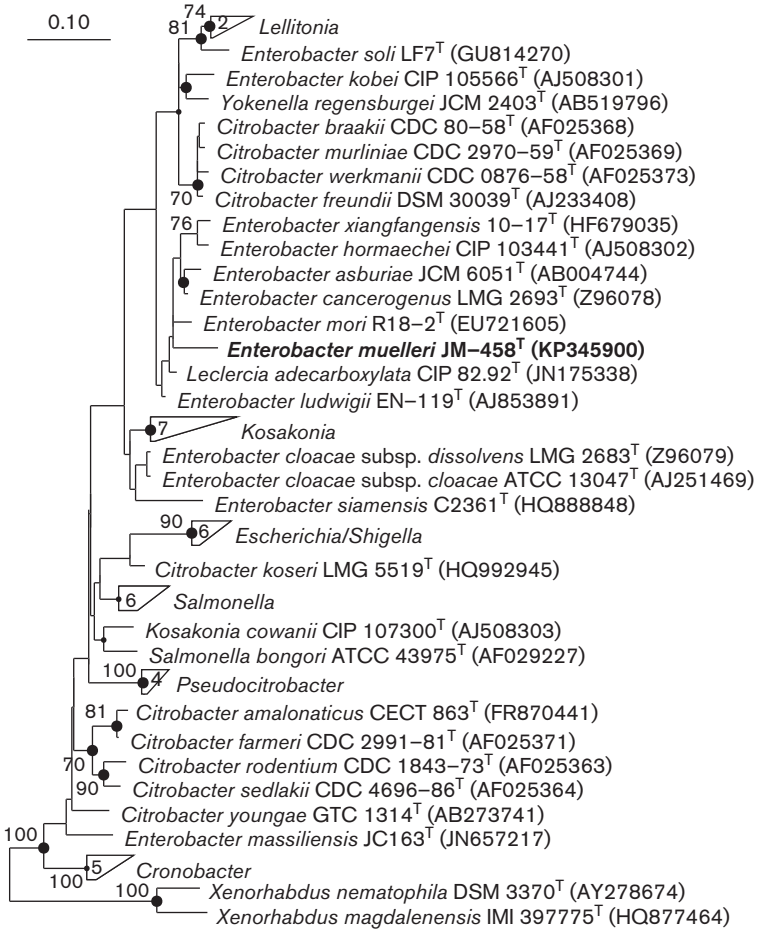

Fig. 1. Phylogenetic affiliation of strain JM-458^T among closest related taxa of the *Enterobacteriaceae* based on nearly full-length 16S rRNA gene sequences. The maximum-parsimony tree was generated in ARB using the LTPs database version 119 (November 2014) and based on nucleotide positions 82–1401 (according to the *E. coli* numbering system; Brosius *et al.*, 1978). Type strain sequences of *Xenorhabdus nematophila* and *Xenorhabdus magdalenensis* were used as the outgroup. Numbers at nodes represent bootstrap values (≥70 %) based on 100 replications. Numbers given in clustered branches represent the number of sequences included in the cluster. Nodes marked with filled circles were also present in the maximum-likelihood tree with ≥70 % bootstrap support. Bar, 0.1 substitutions per nucleotide position. The tree was originally calculated including all type strain sequences of the *Enterobacteriaceae*. Those that did not cluster directly with strain JM-458^T were removed from the tree without changing the overall tree topology.

(1998) (except that for nick translation 2 μg of DNA was labelled during 3 h of incubation at 15 °C). Strain JM-458^T showed low DNA–DNA relatedness to *E. mori* LMG 25706^T (29 %, reciprocal 25 %), *E. hormaechei* EN 314^T (24 %, reciprocal 43 %), *E. ludwigii* EN-119^T (16 %, reciprocal 17 %) and *Enterobacter cloacae* subsp. *cloacae* DSM 30054^T (9 %), confirming the distinct species status of the new isolate.

The analysis of cellular fatty acid profiles of whole-cell hydrolysates was done as described previously (Kämpfer & Kroppenstedt, 1996) and revealed a profile typical of the genus *Enterobacter* with the following most abundant fatty acids: $C_{16:1}$, $C_{16:0}$ cyclo, iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ and $C_{18:1}\omega7c$. The complete fatty acid pattern of strain JM-458^T is shown in Table 2 in comparison with those of the type strains of the most closely related *Enterobacter* species.

On the basis of the results of this polyphasic study, strain JM-458^T is considered to represent a novel species of the genus *Enterobacter*, for which the name *Enterobacter muelleri* sp. nov. is proposed.

Description of *Enterobacter muelleri* sp. nov.

Enterobacter muelleri (muel′le.ri N.L. gen. n. *muelleri* named after Hans Emil Müller, a German microbiologist

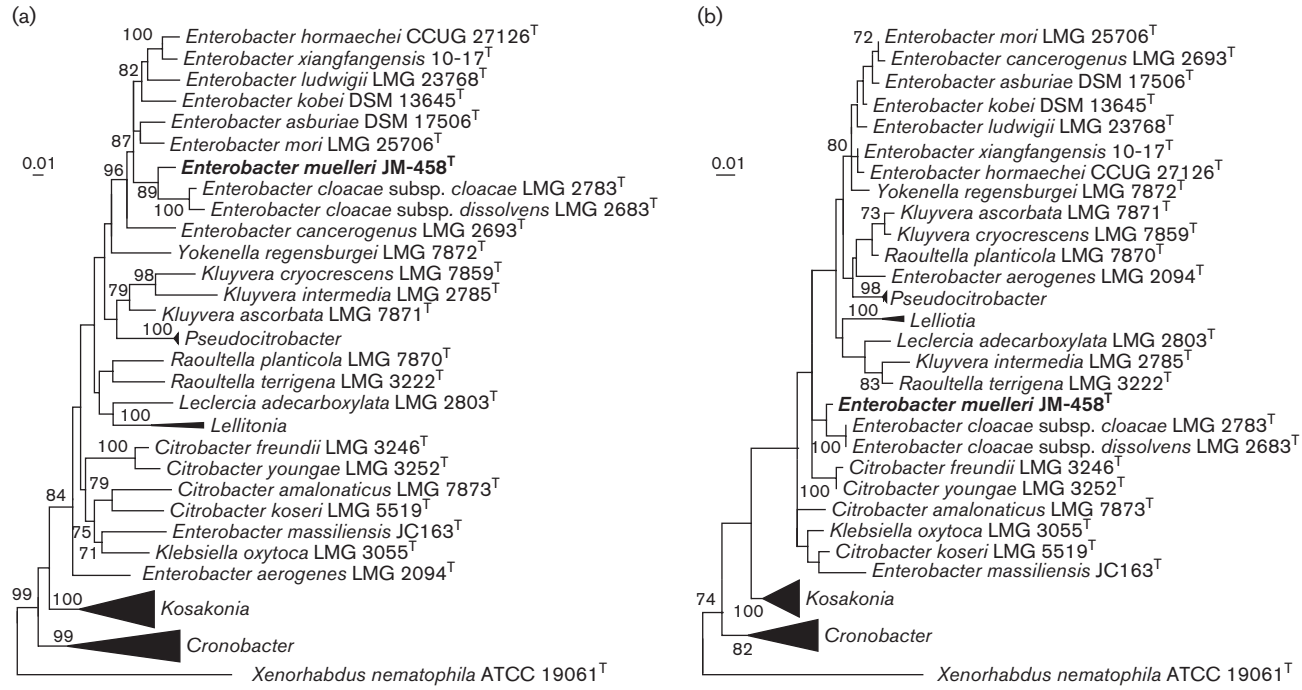

Fig. 2. Phylogenetic affiliation of strain JM-458^T among closest related taxa of the *Enterobacteriaceae* based on concatenated partial *rpoB*, *gyrB*, *infB* and *atpD* nucleotide (a) and amino acid (b) sequences (MLSA). Accession numbers for individual genes are given in Table S1. Phylogenetic trees were calculated in MEGA5 using the maximum-likelihood method with the GTR + G + I model for nucleotide-based analysis and the JTT + G + I model for amino-acid-based analysis. Both phylogenetic trees were based on 100 replications (bootstraps). The concatenated sequence consisted of 635, 739, 607 and 642 nt and 211, 246, 202 and 214 aa sequence positions, respectively. Bootstrap values <70 % are not shown. *Xenorhabdus nematophila* ATCC 19061^T was used as an outgroup. Bars, 0.01 nt or amino acid substitutions. The tree was originally calculated including additional type strain sequences of the *Enterobacteriaceae*. Those that did not cluster directly with strain JM-458^T were removed from the tree without changing the overall tree topology.

who has made a significant contribution to our knowledge of *Enterobacteriaceae*).

Beige colonies (diameter about 2 mm) are formed on NA at 28 °C. Cells are Gram-stain-negative, motile, non-spore-forming rods, approximately 1 μm in width and 2 μm in length. Aerobic, and oxidase- and catalase-negative. Good growth occurs after 48 h on NA, brain heart infusion agar, TSA, R2A agar and MacConkey agar (all Oxoid) at 28 °C. Growth occurs on NA at 8–45 °C, but not at 4 or 50 °C. Cells grow at 28 °C in the presence of 1.0–7.0 % NaCl [weak with 8 and 9 % (w/v); no growth with 10 % (w/v)] as an additional ingredient of TSB. Produces acid from D-glucose, sucrose, L-arabinose, cellobiose, D-mannitol, *i*-inositol, salicin, D-sorbitol, maltose, D-mannose, trehalose, D-xylose and methyl α-D-glucoside, but not from adonitol, D-arabitol, dulcitol, erythritol, lactose, melibiose, raffinose or L-rhamnose. Negative for urease activity, indole production, lysine decarboxylase, production of brown diffusible pigments on tyrosine agar, hydrogen sulphide production and alkalinization of malonate; positive for activity of arginine dihydrolase, ornithine decarboxylase (weak), alkalinization of citrate (Simmons), aesculin hydrolysis and β-galactosidase (ONPG). The following compounds are utilized as a sole source of carbon: acetate, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, D-glucose, maltose, D-mannose, D-fructose, trehalose, glycerol, D-mannitol, D-ribose, sucrose, salicin, D-xylose, *i*-inositol, D-sorbitol, *cis*-aconitate, *trans*-aconitate, fumarate, DL-lactate, pyruvate, citrate, mesaconate, L-alanine, L-serine and L-histidine. The following compounds are not utilized as a sole source of carbon: α-melibiose, L-rhamnose, adonitol, maltitol, putrescine, propionate, adipate, 4-aminobutyrate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, mesaconate, 2-oxoglutarate, suberate, β-alanine, L-aspartate, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates *p*-nitrophenyl-β-D-glucopyranoside (weak), *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-xylopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl-phenylphosphonate, 2-deoxythymidine-2′-*p*-nitrophenyl phosphate, L-alanine-*p*-nitroanilide, γ-L-glutamate-*p*-nitroanilide and L-proline-*p*-nitroanilide are hydrolysed; *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucuronide and

Table 2. Cellular fatty acid composition of strain JM-458^T and the type strains of related species of the genus *Enterobacter*

Strains: 1, JM-458^T; 2, *E. mori* R18-2^T; 3, *E. asburiae* ATCC 35953^T; 4, *E. cancerogenus* LMG 2693^T; 5, *E. hormaechei* NBRC 105718^T; 6, *E. xiangfangensis* 10-17^T; 7, *E. cloacae* subsp. *cloacae* NBRC 13535^T; 8, *E. cloacae* subsp. *dissolvens* LMG 2683^T. Data for strain JM-458^T are from this study. Data for strains 6–8 are from Gu *et al.* (2014) and other data are from Zhu *et al.* (2011), obtained under the same conditions. Fatty acids representing less than 0.1 % in all strains were omitted. ECL, equivalent chain-length; ECL deviation <0.005; –, not found.

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$	–	–	0.1	–		–	–	0.1
$C_{11:0}$	–	–	–	–	0.1	–	–	–
$C_{12:0}$	3.8	2.1	3.5	4.3	2.5	2.3	2.5	4.5
$C_{13:0}$	–	–	0.1	1.2	1.2	0.4	0.6	0.7
$C_{14:0}$	5.2	7.0	7.7	7.2	8.4	9.7	6.7	8.8
$C_{14:0}$ 2-OH	–	0.9	–	–	–	–	–	–
$C_{15:0}$	–	–	0.6	0.7	–	–	–	–
anteiso-$C_{15:0}$	–	–	–	–	–	–	–	0.9
$C_{15:0}$ 3-OH	0.3	0.2	0.4	0.4	0.4	–	0.2	0.3
iso-$C_{15:0}$ 3-OH	–	–	2.9	2.6	–	–	–	10.6
$C_{16:0}$	29.5	28.8	22.4	24.1	21.8	30.2	29.3	23.2
$C_{16:1}\omega5c$	–	0.2	–	–	0.2	–	0.2	–
$C_{17:0}$	1.8	2.8	1.1	3.7	4.9	2.0	3.4	4.2
$C_{17:0}$ cyclo	18.3	18.3	9.7	14.8	21.1	21.7	20.9	6.7
$C_{17:1}\omega8c$	0.5	0.2	0.1	0.6	0.4	–	–	0.3
$C_{18:0}$	1.7	0.3	0.1	1.1	0.4	0.5	0.5	–
$C_{18:1}\omega7c$	11.5	17.8	15.8	17.5	20.6	15.9	14.3	18.3
$C_{19:0}$ cyclo $\omega8c$	1.4	1.1	1.2	0.5	4.4	5.8	6.1	–
Summed feature 1*	–	0.5	1.1	1.3	1.5	0.3	0.6	3.0
Summed feature 2*	3.4	5.4	10.2	9.1	6.2	6.8	6.9	13.0
Summed feature 3*	22.3	13.1	25.9	23.2	5.7	4.5	6.5	23.0
Unknown ECL 14.502	0.3	0.4	–	–	–	–	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1, $C_{13:0}$ 3-OH/iso-$C_{15:1}$ I; summed feature 2, iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH; summed feature 3, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

bis-*p*-nitrophenyl-phosphoryl-choline are not hydrolysed. The major cellular fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ and $C_{18:1}\omega7c$.

The type strain, JM-458^T (=DSM 29346^T=CIP 110826^T=LMG 28480^T=CCM 8546^T), was isolated from the rhizosphere of 1-week-old corn (*Zea mays*, cultivar 'Sweet Belle') grown at the Plant Breeding unit facility at the E.V. Smith Research Center in Tallassee (Elmore county), AL, USA.

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