Elizabethkingia endophytica sp. nov., isolated from Zea mays and emended description of Elizabethkingia anophelis Kämpfer et al. 2011

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A slightly yellow bacterial strain (JM-87T), isolated from the stem of healthy 10 day-old sweet corn (Zea mays), was studied for its taxonomic allocation. The isolate revealed Gram-stain-negative, rod-shaped cells. A comparison of the 16S rRNA gene sequence of the isolate showed 99.1, 97.8, and 97.4 % similarity to the 16S rRNA gene sequences of the type strains of Elizabethkingia anophelis, Elizabethkingia meningoseptica and Elizabethkingia miricola, respectively. The fatty acid profile of strain JM-87T consisted mainly of the major fatty acids C15:0 iso, C17:0 iso 3-OH, and C19:0 iso 2-OH/C16:1ω7c/t. The quinone system of strain JM-87T contained, exclusively, menaquinone MK-6. The major polyamine was sym-homospermidine. The polar lipid profile consisted of the major lipid phosphatidylethanolamine plus several unidentified aminolipids and other unidentified lipids. DNA–DNA hybridization experiments with E. meningoseptica CCUG 214T (=ATCC 13253T), E. miricola KCTC 12492T (=GTC 862T) and E. anophelis R26T resulted in relatedness values of 17 % (reciprocal 16 %), 30 % (reciprocal 19 %), and 51 % (reciprocal 54 %), respectively. These DNA–DNA hybridization results, in addition to some differentiating biochemical properties, clearly indicate that strain JM-87T is a representative of a novel species, for which the name Elizabethkingia endophytica sp. nov. is proposed. The type strain is JM-87T (=CIP 110885T =LMG 28604T =CCM 8570T).

Initially the genus Elizabethkingia was proposed by Kim et al. (2005), who described the two species Elizabethkingia meningoseptica and Elizabethkingia miricola mainly on the basis of 16S rRNA gene sequence similarity studies. Strains of these species have been shown, in earlier studies, to represent a separate lineage from the type strains of the Chryseobacterium–Bergeyella–Riemerella branch within the family Flavobacteriaceae (90.7–93.9 % similarities), which was also supported by phenotypic differences (Bernardet et al., 2006). In 2011, an additional species Elizabethkingia anophelis was described, which was isolated from the midgut of the mosquito Anopheles gambiae (Kämpfer et al., 2011).

Strain JM-87T was isolated as an endophyte from the healthy internal stem tissue of 10 day-old sweet corn (Zea mays, cultivar ‘Sweet Belle’) grown at the Plant Breeding unit facility at the E.V. Smith Research Center in Tallassee, Alabama USA.

The cultural and morphological characteristics were determined from fresh cultures grown on tryptic soy agar (TSA; Oxoid) at 28 °C. For Gram staining, a modified method of Gerhardt et al. (1994) was used and the motility was determined with light microscopy (Axiohot2, Zeiss; 1000 × magnification) of cells grown for 3 days in tryptic soy broth (TSB; Oxoid) at 28 °C. Temperature-dependent growth was tested at 4, 11, 30, 37 and 45 °C on nutrient agar. NaCl tolerance was investigated at different concentrations of NaCl [0.5–8.0 % (w/v)] in TSB.

The strain showed Gram-stain-negative behaviour and formed visible yellowish colonies (diameter approximately 2 mm) within 48 h at 28 °C. No growth was observed below 15 °C or above 45 °C. All strains grew very slowly at 45 °C and at an NaCl concentration of 1–2 % (w/v).

The colonies of the isolate had a translucent, glistening appearance with entire edges. A yellow pigment of the flexirubin type (KOH method according to Reichenbach, 1992) was produced on nutrient agar. Oxidase activity

Abbreviations: AL, aminolipid; DPG, diphosphatidylglycerol; Lphospholipid; PE, phosphatidylethanolamine; PI, phosphatidylinositol

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain JM-87T is KP113704.
was positive when tested with the oxidase reagent (bioMérieux). Cells of the strains were non-motile and rod-shaped (approximately 1 μm wide and 2 μm long); no spores could be detected. Strain JM-87T grew well on nutrient agar, brain heart infusion agar, R2A agar and TSA, but not on MacConkey agar (Oxoid).

Physiological and biochemical characterizations (96-well plate test system) and some additional biochemical tests were carried out, as described by Kämpfer et al. (1991) and Kämpfer (1990). The following additional biochemical tests were performed: production of hydrogen sulphide using lead acetate paper and triple-sugar-iron methods; the indole reaction with Ehrlich’s and Kovacs’ reagent; the activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, DNase, β-galactosidase (ONPG) and urease; the hydrolysis of casein, gelatin (plate method); starch and tyrosine (Smibert & Krieg, 1994).

As reported previously for many other species of the genus Elizabethkingia, the strain utilized very few carbon sources, but was able to hydrolyse some chromogenic substrates. The biochemical/physiological data are given in Table 1 and in the species description.

Isolate JM-87T was maintained and subcultivated on nutrient agar (NA; Oxoid) at 30 °C for 48 h and subsequently analysed for its 16S rRNA gene sequence, fatty acid methyl ester composition of the whole cell hydrolysate, quinone and polar lipid pattern, further phenotypic characteristics, and DNA–DNA relatedness to species most closely related on the basis of 16S rRNA gene sequence similarities.

The phylogenetic analysis was based on nearly full-length 16S rRNA gene sequences. The sequenced 16S rRNA gene fragment of strain JM-87T was a continuous stretch of 1428 nt spanning gene positions 17 to 1471 according to the Escherichia coli rrnB numbering (Brosius et al., 1981). A first phylogenetic placement and pairwise sequence similarities to closest related type strains was obtained by BLAST analysis against the EzTaxon type strain database (Kim et al., 2012). Phylogenetic trees were reconstructed with the software package ARB release 5.2 (Ludwig et al., 2004) and the ‘All-Species Living Tree’ Project (LTP; Yarza et al., 2008) database release LTPs115 (March 2013). The sequence of JM-87T was aligned using the SINA online tool v. 1.2.11; Pruesse et al., 2012) and implemented into the LTP database. The alignment of sequences used for tree reconstruction was checked manually. Pairwise 16S rRNA gene sequence similarities were calculated using the ARB neighbour-joining tool without an evolutionary substitution model. Phylogenetic trees were reconstructed with different treeing methods, including the maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, the maximum-parsimony method using dnapsars version 3.6 (Felsenstein, 2005) and the neighbour-joining method using ARB neighbour-joining with the Jukes-Cantor correction model (Jukes & Cantor, 1969). All trees were calculated with 100 resamplings (bootstrap analysis; Felsenstein, 1985) and based on 16S rRNA gene sequence positions between gene termini 82 to 1394 (according to E. coli rrnB numbering; Brosius et al., 1981).

Pairwise sequence similarity calculations showed highest sequence similarities to the type strains of E. anophelis (99.1%) followed by E. meningoseptica (97.8%) and E. miricola (97.4%). Sequence similarities to all type strains of the next most closely related genera were below 95%. Phylogenetic trees showed, independently of the treeing method applied, placement of strain JM-87T into the monophyletic cluster of the genus Elizabethkingia including the three species listed above within the Flavobacteriaceae (Fig. 1).

Cellular fatty acid analysis was performed for strain JM-87T with the same method as for type strains of E. meningoseptica, E. miricola and E. anophelis after extraction of whole cell hydrolysates according to Kämpfer & Kroppenstedt (1996) using the Sherlock Microbial Identification System (Sherlock MIDI software version 2.11 and TSBA peak-naming table version 4.1). The strains were grown until they reached the end of the exponential phase (controlled visually by assessment of the colony sizes) and subsequently harvested from the agar plates.

The fatty acid profile of isolate JM-87T was characterized by containing large amounts of C15:0 iso, C17:0 iso 3-OH, and summed feature 4 (C15:0 iso 2-OH / C16:1ω7c t), which is in congruence with results obtained in previous studies.

Table 1. Characteristics that differentiate species of the genus Elizabethkingia

<table>
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<tr>
<th>Characteristic</th>
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<td>Citrate utilization</td>
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<td>Melibiose</td>
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<td>−</td>
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<td>Growth on MacConkey agar</td>
<td>−</td>
<td>−</td>
<td>+ (v)</td>
<td>(+)</td>
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<td>Hydrolysis of urea</td>
<td>+</td>
<td>−</td>
<td>− (−)</td>
<td>+ (+)</td>
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*Bernardet et al. (2006) reported, that for E. meningoseptica acid production from cellulobiose was found to vary between references, and growth on MacConkey agar was found to be strain-dependent. These findings were based on the study of a large number of E. meningoseptica strains.
JM-87T was grown on 3.3-mines, respiratory quinones and polar lipids, strain for analysis of the peptidoglycan diamino acids, polya-obtained is presented in Table 2.

The presence of \textit{meso}-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan is a characteristic shared among species of the very closely related genus \textit{Chryseobacterium}. The polyamine pattern with the major compound \textit{sym}-homospermidine and quinone system menaquinone MK-6 are in line with other representatives of the \textit{Flavobacteriaceae}, including \textit{E. meningoseptica}, \textit{E. miricola} and \textit{E. anophelis} (Ka¨mpfer et al., 2011). However, the absence of PE and presence of DPG and PI (as reported for the three species of the genus \textit{Elizabethkingia} with validly published names \textit{E. meningoseptica}, \textit{E. miricola} and \textit{E. anophelis}) was not found and for this reason, lipid analyses were repeated with the type strains of all three of these species. This was also carried out because no reliable report exists on the absence of PE and presence of phosphatidylethanolamine and DPG in species of the \textit{Flavobacteriaceae}. Furthermore, the presence

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Fig. 1. Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic placement of strain JM-87\textsuperscript{T} into the genus \textit{Elizabethkingia} within the \textit{Flavobacteriaceae}. The tree was generated in ARB using RAxML (GTR-GAMMA; rapid bootstrap analysis with 100 replications) and based on 16S rRNA gene positions 82 to 1394 (Brosius et al., 1981). Numbers at the nodes represent bootstrap values >70\%. Asterisks indicate those nodes which were also supported with high bootstrap values in the maximum-parsimony tree. The type strain of \textit{Wandonia haliotis} was used to root the tree (outgroup). Bar, 0.10 nucleotide substitutions per nucleotide position.

(Fig. 2A) was composed of phosphatidylethanolamine (PE), four unidentified aminolipids (ALs), two unidentified glycolipids (GLs) and four polar lipids (Ls), which were only detectable after staining for total lipids. The presence of \textit{meso}-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan is a characteristic shared by the majority of Gram-negative bacteria, including the \textit{Bacteroidetes}. The polyamine pattern with the major compound \textit{sym}-homospermidine and quinone system menaquinone MK-6 are in line with other representatives of the family \textit{Flavobacteriaceae}, including \textit{E. meningoseptica} and species of the very closely related genus \textit{Chryseobacterium} (Hamana & Matsuzaki, 1990; Vandamme et al., 1994).
of several unidentified ALs is widespread among members of this family, including species of the closely related genus Chryseobacterium (Montero-Calasanz et al., 2013; Nguyen et al., 2013; Kämpfer et al., 2003, 2014; Kook et al., 2014). The polar lipid profiles of these three species (Figs 2b–d) were found to be very similar to each other and to that of strain JM-87T. They shared the presence of PE, four unidentified ALs and two major unidentified Ls (L2, L4) and did not contain DPG, PI or a characteristic phospholipid, as reported initially (Kämpfer et al., 2011). These results indicate that the polar lipid profiles of species of the genus Elizabethkingia are very similar to those of species of the genus Chryseobacterium.

DNA–DNA hybridization experiments were performed between strain JM-87T and the type strains of the three species of the genus Elizabethkingia according to the method of Ziemke et al. (1998) (except for that in nick translation 2 μg DNA was labelled during 3 h of incubation at 15 °C). These experiments with E. meningoseptica CCUG 214T, E. miricola KCTC 12492T and E. anophelis R26T resulted in DNA relatedness values of 17% (reciprocal 16%), 30% (reciprocal 19%), and 51% (reciprocal 54%), respectively.

Although the chemotaxonomic data demonstrated a high similarity of strain JM-87T to the type strains of all three species of the genus Elizabethkingia with validly published names, hybridization experiments and the physiological and chemotaxonomic data allow strain JM-87T to be clearly separated from them. On the basis of the results reported we propose a novel species name, Elizabethkingia endophytica sp. nov., for strain JM-87T.

Furthermore, an emended description of Elizabethkingia anophelis is required because this species was reported to contain, as a characteristic trait, the phospholipids DPG and PI with no ALs.

**Emended description of Elizabethkingia anophelis Kämpfer et al. 2011**

Elizabethkingia anophelis (a.no.phe’lis. N. L. gen. n. anopheliς of/from a mosquito of the genus Anopheles, as the type strain was isolated from the midgut of Anopheles gambiae).

In contrast to the original description of the species (Kämpfer et al. 2011), it does not contain DPG, PI and a characteristic phospholipid. Instead, PE, four unidentified ALs and two major characteristic polar lipids are detected.

**Description of Elizabethkingia endophytica sp. nov.**

Elizabethkingia endophytica (en.do.phy’ti.ca. Gr. pref. *endo* within; Gr. n. *phyton* plant; L. fem. suff. -ica adjectival suffix used with the sense of belonging to; N.L. fem. adj. *endophytica* within plant, endophytic, pertaining to the original isolation from plant tissue).

Gram-stain-negative, non-motile, non-spore-forming rods, approximately 1 μm in width and 2 μm in length. Aerobic, and oxidase and catalase-positive. Good growth occurs after 48 h on NA, R2A agar and TSA (all Oxoid) at 15–45 °C. No growth occurs on MacConkey agar (Oxoid) at 28 °C. Unable to grow at temperatures below 15 °C and above 45 °C. Colonies on NA are smooth, yellowish,
circular, translucent and glistening with entire edges. The non-diffusible and non-fluorescent yellow pigment is not of the flexirubin type (KOH test negative). Arginine dihydrolase, ornithine decarboxylase, lysine decarboxylase, urease, DNase, gelatin, tyrosine, the hydrolysis of starch and H₂S production are negative.

Acid is produced from D-glucose, lactose (weakly), D-mannitol, maltose, trehalose, and cellobiose (weakly). No acid is produced from adonitol, L-arabinose, D-arabitol, dulcitol, erythritol, myo-inositol, melibiose, α-methyl-D-glucoside, raffinose, salicin, L-rhamnose, sucrose, D-sorbitol, and D-xylene. Aesculin hydrolysis, indole production and β-galactosidase activity (ONPG) are positive. Urease activity, hydrolysis of casein, gelatin, starch, DNA and tyrosine, hydrogen sulphide production, activity of arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, and utilization of malonate are negative. The following compounds are utilized as sole sources of carbon: D-fructose, D-glucose, maltose, D-mannose and D-mannitol. The following compounds are not utilized as a sole source of carbon: N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, glycerol, maltitol, α-melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, D-xylene, adonitol, myo-inositol, D-sorbitol, putrescine, acetate, propionate, cis-aconitate, trans-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

Elizabethkingia endophytica sp. nov.

Fig. 2. Total polar lipid profiles of: (a) strain JM-87T; (b) E. meningoseptica CCUG 214T; (c) E. anophelis R26T and (d) E. miricola KCTC 12492T. Staining was with molybdatophosphoric acid. PE, phosphatidylethanolamine; AL1–4, unidentified aminolipids; L1–14, unidentified polar lipids; GL1–2, unidentified glycolipids.

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The chromogenic substrates p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-α-D-glucopyranoside, bis-p-nitrophenyl-phosphate, bis-p-nitrophenyl-phenyl-phosphonate, bis-p-nitrophenyl-phosphoryl-choline, 2-deoxothymidine-2′-p-nitrophenylphosphate, l-alanine-p-nitroaniline, γ-L-glutamate-p-nitroanilide and l-proline-p-nitroanilide are hydrolysed, but p-nitrophenyl-β-D-xlyopyranoside and p-nitrophenyl-β-D-glucuronide are not. The major cellular fatty acids are C15:0 iso, C17:0 iso 3-OH and summed feature 4 (C15:0 iso 2-OH / C16:0 7c/t). The diagnostic diamino acid of the peptidoglycan is meso-diaminopimelic acid and in the polyamine pattern sym-homospermidine is predominant. The quinone system is menaquinone MK-6. The polar lipid profile consists of PE, four unidentified ALs, two unidentified glycolipids and four polar lipids.

The type strain is JM-87T (=CIP 110885T =LMG 28604T =CCM 8570T), isolated in 1990 as an endophyte from the healthy internal stem tissue of 10 day-old corn (Zea mays, cultivar ‘Sweet Belle’) grown at the Plant Breeding unit facility at the E.V. Smith Research Center in Tallasee (Macon county), Alabama USA.

Acknowledgements

We are grateful to Gundula Will, Maria Sowinsky and Katja Grebing for excellent technical assistance.

References


