# *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-87$^T$), 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-87$^T$ consisted mainly of the major fatty acids $C_{15:0}$ iso, $C_{17:0}$ iso 3-OH, and $C_{15:0}$ iso 2-OH/$C_{16:1}\omega7c/t$. The quinone system of strain JM-87$^T$ 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 214$^T$ (=ATCC 13253$^T$), *E. miricola* KCTC 12492$^T$ (=GTC 862$^T$) and *E. anophelis* R26$^T$ 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-87$^T$ is a representative of a novel species, for which the name *Elizabethkingia endophytica* sp. nov. is proposed. The type strain is JM-87$^T$ (=CIP 110885$^T$= LMG 28604$^T$=CCM 8570$^T$).

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-87$^T$ 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 (Axiophot2, 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-87$^T$ 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-87$^T$ 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, $\beta$-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-87$^T$ 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-87$^T$ was a continuous stretch of 1428 nt spanneding 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-87$^T$ 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 DNAPARS 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-87$^T$ 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-87$^T$ 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-87$^T$ was characterized by containing large amounts of $C_{15:0}$ iso, $C_{17:0}$ iso 3-OH, and summed feature 4 ($C_{15:0}$ iso 2-OH / $C_{16:1}\omega7c/t$), which is in congruence with results obtained in previous studies

**Table 1.** Characteristics that differentiate species of the genus *Elizabethkingia*

Strains: 1, JM-87$^T$; 2, *E. anophelis* R26$^T$; 3, *E. meningoseptica* CCUG 214$^T$; 4, *E. miricola* KCTC 12492$^T$. Acid production from arabinose, raffinose, salicin, sucrose and xylose were negative for all strains tested. Acid production from glucose, lactose, maltose, mannitol and trehalose was positive for all strains. +, Positive; v, variable; −, negative; all data are from this study and Kämpfer *et al.* (2011), except those shown in parentheses (from Kim *et al.*, 2005). Only characteristics that differ between the three strains are shown.

| Characteristic | 1 | 2 | 3* | 4 |
|---|---|---|---|---|
| Citrate utilization | − | − | − (v) | +(+) |
| Acid production from: | | | | |
| Cellobiose | − | + | − (−) | − (−) |
| Melibiose | + | − | + | − |
| Growth on MacConkey agar | − | − | + (v) | +(+) |
| Hydrolysis of urea | + | − | − (−) | +(+) |

*Bernardet *et al.* (2006) reported, that for *E. meningoseptica* acid production from cellobiose 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.

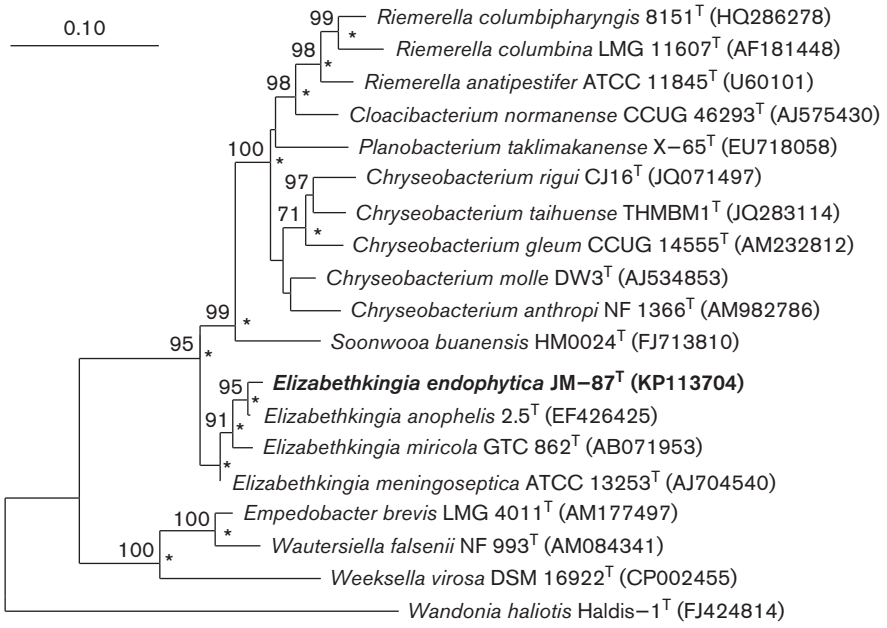


**Fig. 1.** Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic placement of strain JM-87$^T$ into the genus *Elizabethkingia* within the *Flavobacteriaceae*. The tree was generated in ARB using RAxML (GTR-GAMMA; rapid bootstrap analysis with 100 replications) 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 *Wandonia haliotis* was used to root the tree (outgroup). Bar, 0.10 nucleotide substitutions per nucleotide position.

(Kämpfer *et al.*, 2011). The detailed pattern of fatty acids obtained is presented in Table 2.

For analysis of the peptidoglycan diamino acids, polyamines, respiratory quinones and polar lipids, strain JM-87$^T$ was grown on 3.3× PYE medium [1.0 % (w/v) peptone from casein, 1.0 % (w/v) yeast extract, pH 7.2]. Biomass subjected to polyamine analysis was harvested at the late exponential growth phase and extracted as described by Busse & Auling (1988). HPLC analysis was carried out as described by Busse *et al.* (1997) using the equipment reported by Stolz *et al.* (2007). Biomass subjected to analyses of the peptidoglycan diamino acid, quinones and polar lipids was harvested at the stationary growth phase. The analysis of the diamino acid was carried out according to Schumann (2011). Quinones and polar lipids were extracted and analysed by applying an integrated procedure (Tindall, 1990a, b; Altenburger *et al.*, 1996). The polyamine pattern consisted of the predominant compound *sym*-homospermidine [26.8 μmol (g dry weight)$^{-1}$], minor amounts of spermine, spermidine, cadaverine and putrescine [each 0.1 μmol (g dry weight)$^{-1}$] and trace amounts of *sym*-norspermidine [<0.1 μmol (g dry weight)$^{-1}$]. The peptidoglycan diamino acid was identified to be *meso*-diaminopimelic acid. The quinone system contained only menaquinone MK-6. The polar lipid profile (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 *meso*-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan is a characteristic shared by the majortiy of Gram-negative bacteria, including the *Bacteroidetes*. The polyamine pattern with the major compound *sym*-homospermidine and quinone system menaquinone MK-6 are in line with other representatives of the family *Flavobacteriaceae*, including *E. meningoseptica* and species of the very closely related genus *Chryseobacterium* (Hamana & Matsuzaki, 1990; Vandamme *et al.*, 1994). The presence of PE, several ALs, Ls and the absence of diphosphatidylglycerol (DPG) and phosphatidylinositol (PI) clearly distinguished JM-87$^T$ from other members of the genus *Elizabethkingia* (Kämpfer *et al.*, 2011). However, the absence of PE and presence of DPG and PI [as reported for the three species of the genus *Elizabethkingia* with validly published names (*E. meningoseptica, E. miricola* and *E. anophelis*)(Kämpfer *et al.*, 2011)] 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 phosphatidylglycerol and DPG in species of the *Flavobacteriaceae*. Furthermore, the presence

**Table 2.** Cellular fatty acids (as a percentage of the totals) of species of the genus *Elizabethkingia*

Strains: 1, JM-87$^T$; 2, *E. anophelis* R26$^T$; 3, *E. meningoseptica* CCUG 214$^T$; 4, *E. miricola* KCTC 12492$^T$. All data are from this study and Kämpfer *et al.* (2011), except for those shown in parentheses, which are from Kim *et al.* (2005). Values in parentheses were derived from $n=5$ (2) and $n=2$ (3) strains. Means ± SD are given (from Kim *et al.*, 2005). TR, Trace amounts (less than 1.0 %); ECL, equivalent chain-length (i.e. the identity of the fatty acid is unknown). Fatty acids amounting to <1 % of the total fatty acids in the four strains are not shown.

| Fatty Acid | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $C_{13:0}$ iso | 1.6 | 1.8 | 2.8 (1.3 ± 0.3) | 2.1 (2.0 ± 0.5) |
| Unknown ECL 13.566 | 6.8 | 6.8 | 6.3 (1.9 ± 0.3) | 4.9 (1.5 ± 0.2) |
| $C_{15:0}$ iso | 43.8 | 45.8 | 40.8 (43.9 ± 2.0) | 44.7 (46.4 ± 2.2) |
| $C_{15:0}$ iso 3-OH | 3.2 | 2.9 | 3.1 (2.8 ± 0.3) | 3.6 (3.0 ± 0.6) |
| $C_{15:0}$ anteiso | 1.8 | 1.8 | 3.9 (1.1 ± 0.8) | 1.5 (1.0 ± 0.6) |
| $C_{16:0}$ | TR | TR | 1.8 (TR) | 1.1 (1.2 ± 0.1) |
| $C_{16:0}$ 3-OH | 2.5 | 2.1 | 3.4 (2.6 ± 0.4) | 2.4 (3.0 ± 0.6) |
| $C_{16:0}$ iso 3-OH | TR | TR | 1.3 (TR) | TR (TR) |
| Unknown ECL 16.580 | 1.4 | 1.2 | 1.1 (1.6 ± 0.1) | 1.4 (1.3 ± 0.6) |
| $C_{17:0}$ iso 3-OH | 13.8 | 12.8 | 12.4 (14.6 ± 1.0) | 14.6 (15.3 ± 0.2) |
| $C_{17:1}$ iso$\omega$9*c* | 3.8 | 4.2 | 4.0 (7.8 ± 1.3) | 4.5 (6.6 ± 0.2) |
| Summed feature 4* | 15.8 | 15.6 | 14.2 (19.6 ± 1.0) | 15.7 (17.0 ± 1.3) |

*Summed feature 4 contains $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega$7*c*/*t*.

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-87$^T$. 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-87$^T$ 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 214$^T$, *E. miricola* KCTC 12492$^T$ and *E. anophelis* R26$^T$ 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-87$^T$ 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-87$^T$ 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-87$^T$.

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* Kampfer *et al.* 2011

*Elizabethkingia anophelis* (a.no.phe'lis. N. L. gen. n. *anophelis* 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,

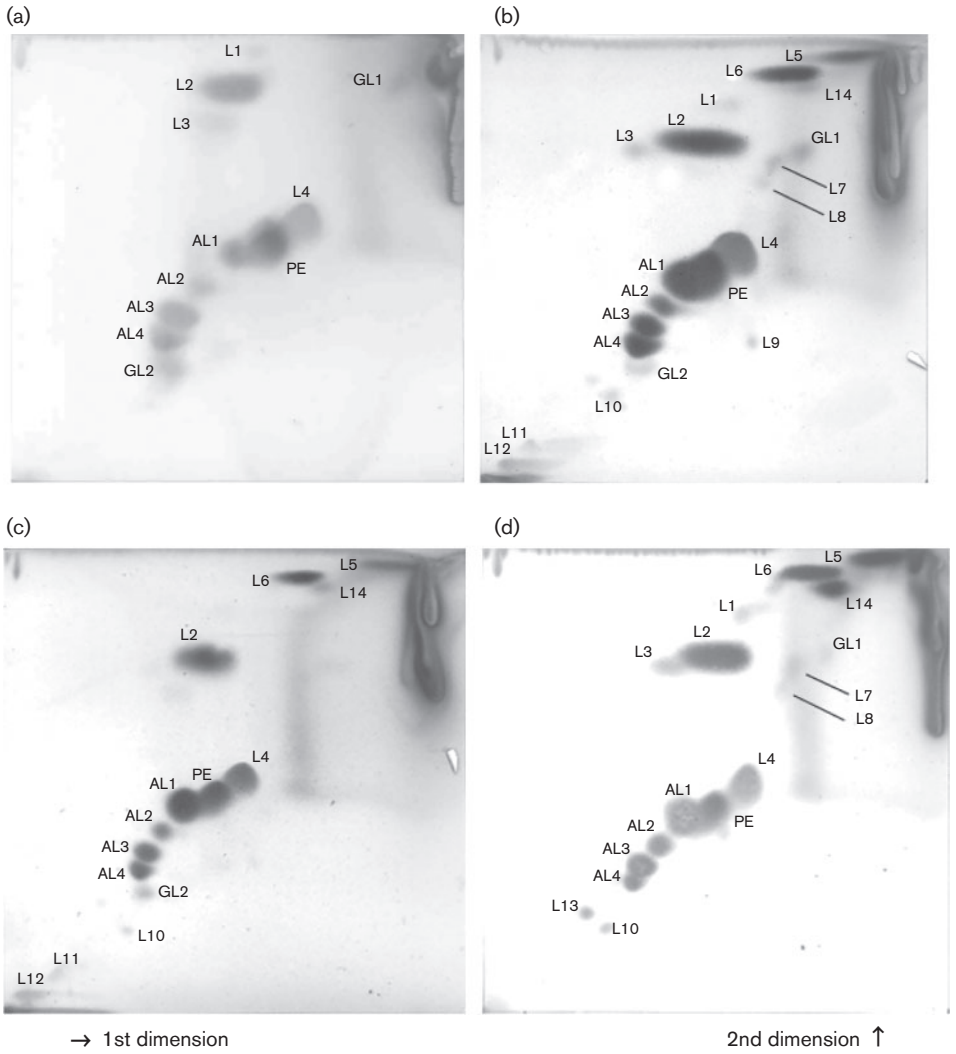


**Fig. 2.** Total polar lipid profiles of: (a) strain JM-87$^T$; (b) *E. meningoseptica* CCUG 214$^T$; (c) *E. anophelis* R26$^T$ and (d) *E. miricola* KCTC 12492$^T$. Staining was with molybdatophosphoric acid. PE, phosphatidylethanolamine; AL1–4, unidentified aminolipids, L1–14, unidentified polar lipids; GL1–2, unidentified glycolipids.

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 $H_2S$ 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-xylose. 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-xylose, 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.

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-deoxythymidine-2′-*p*-nitrophenyl-phosphate, L-alanine-*p*-nitroanilide, γ-L-glutamate-*p*-nitroanilide and L-proline-*p*-nitroanilide are hydrolysed, but *p*-nitrophenyl-β-D-xylopyranoside and *p*-nitrophenyl-β-D-glucuronide are not. The major cellular fatty acids are $C_{15:0}$ iso, $C_{17:0}$ iso 3-OH and summed feature 4 ($C_{15:0}$ iso 2-OH / $C_{16:1}\omega7c/t$). 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-87$^T$ (=CIP 110885$^T$=LMG 28604$^T$=CCM 8570$^T$), 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 Tallassee (Macon county), Alabama USA.

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