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ORIGINAL PAPER

Brevibacterium metallicus sp. nov., an endophytic bacterium isolated from roots of *Prosopis laegivata* grown at the edge of a mine tailing in Mexico

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Abstract A Gram-positive, aerobic, nonmotile strain, NM2E3^T was identified as *Brevibacterium* based on the 16S rRNA gene sequence analysis and had the highest similarities to *Brevibacterium jeotgali* SJ5-8^T (97.3 %). This novel bacterium was isolated from root tissue of *Prosopis laegivata* grown at the edge of a mine tailing in San Luis Potosí, Mexico. Its cells were non-spore-forming rods, showing catalase and oxidase activities and were able to grow in LB medium added with 40 mM Cu^{2+}, 72 mM As^{5+} and various other toxic elements. Anteiso-$C_{15:0}$ (41.6 %), anteiso-$C_{17:0}$ (30 %) and iso-$C_{15:0}$ (9.5 %) were the major fatty acids. MK-8(H_2) (88.4 %) and MK-7(H_2) (11.6 %) were the major menaquinones. The DNA G + C content of the strain NM2E3^T was 70.8 mol % (Tm). DNA–DNA hybridization showed that the strain NM2E3^T had 39.8, 21.7 and 20.3 % relatedness with *B. yomogidense* JCM 17779^T, *B. jeotgali* JCM 18571^T and *B. salitolerans* TRM 45^T, respectively. Based on the phenotypic and genotypic analyses, the strain NM2E3^T (=CCBAU 101093^T = HAMBI 3627^T = LMG 8673^T) is reported as a novel species of the genus *Brevibacterium*, for which the name *Brevibacterium metallicus* sp. nov., is proposed.

Keywords Root tissue · *Prosopis laegivata* · Heavy metals · *Brevibacterium metallicus*

Introduction

The genus *Brevibacterium*, a member of the family *Brevibacteriaceae* of the phylum *Actinobacteria*, was first proposed by Breed (1953) with *Brevibacterium linens* as the type species. This species was later emended by Collins et al. (1977). *Brevibacterium* strains have been isolated from diverse environments such as paintings (Heyman et al. 2004), brown alga (Ivanova et al. 2004), deep sea sediment (Bhadra et al. 2008), sea water (Lee 2008), saline soil (Tang et al. 2008), human skin (Roux and Raoult 2009), moth caterpillars (Kati et al. 2010), indoor wall (Kämpfer et al. 2010), salt lakes (Guan et al. 2010), poultry manure (Tonouchi et al. 2013), river water (Kumar et al. 2013) and activated sludge (Cui et al. 2013; Kim et al. 2013) (LSPN bacterio net; http://www.bacterio.net/). Currently, the genus is comprised of 49 species, including the recently described species *Brevibacterium jeotgali* (Choi et al. 2013).

In a study about the diversity of endophytic bacteria associated with endemic plants grown in a heavy metal contaminated region in Mexico, a strain NM2E3^T was isolated from *Prosopis laegivata* root tissue. This bacterium is able to grow in Luria–Bertani (LB) medium supplied with high concentration of heavy metals, such as 40 mM Cu^{2+}, 2 mM Zn^{2+}, 12.5 mM Pb^{2+}, 5.8 mM As^{3+} and 72 mM As^{5+} (our unpublished data). In the present research, the

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taxonomic status of the strain NM2E3^T is described using a polyphasic approach. Taking into account the results, this strain represents to a novel species within the genus *Brevibacterium,* and the name *Brevibacterium metallicus* sp. nov, is proposed. The type strain is NM2E3^T (=CCBAU 101093^T = HAMBI 3627^T = LMG 8673^T).

Materials and methods

Bacterial isolation and maintenance

To isolate the endophytic bacteria, 1 g of thin roots (<0.5 mm in diameter) sampled from *Prosopis laegivata* grown at the edge of a mine tailing at Villa de la Paz in the state of San Luis Potosi (23.7 N, 178.7 W) in Mexico was surface sterilized according to Marquez-Santacruz et al. (2010). Subsequently, 1 g of the sample was macerated in 9 mL of 0.85 % NaCl under aseptic conditions (Barzanti et al. 2007). Aliquots (100 µL) of the tissue extracts (10^{-1} a 10^{-3}) were plated in triplicate on tryptic soya agar (TSA, Difco) (Gardner et al. 1982). The inoculated medium was incubated at 28 °C for one to 14 days (Sun et al. 2010). Colonies were selected according to their morphology and purified by repeatedly streaking on the same medium. Pure colonies were subcultured and maintained on TSA medium at 4 °C and stored at −70 °C in a liquid medium supplied with 50 % (w/v) of glycerol. To confirm that the disinfection process was successful, aliquots of 100 µL of the sterilized water used in the final rinse during the surface sterilization was inoculated on the same medium and incubated under the same conditions.

Sequencing and phylogenetic analysis of 16S rRNA gene

This analysis was performed for 60 endophytic isolates to scan their taxonomic affinities. Genomic DNA was extracted for each isolate from 5 ml of culture in TS broth incubated for 18 h at 28 °C with shaking (150 rpm), using the protocol of Zhou et al. (1995). Briefly, cells were lysed with 50 mg mL^{-1} of lysozyme, 70 µL of 10 % (v/w) SDS in 0.1 × TE and 10 mg mL^{-1} of proteinase K to avoid the difficulty of extraction of DNA from the Gram-positive bacteria. The amplified 16S rRNA gene using the DNA extract as template and the primers fD1 and rD1 (Weisburg et al. 1991) was sequenced according to the protocols described by Sun et al. (2010). The acquired sequences were compared with those in the GenBank database using the program BLAST on the Web site http://blast.ncbi.nlm.nih.gov/Blast.cgi (Altschul et al. 1997). The 16S rRNA gene sequences obtained in this study were aligned using CLUSTAL X (2.0) software (Thompson et al. 1997) together with the closely related sequences extracted by BLAST searching and were manually edited with SEAVIEW software (Galtier et al. 1996). Phylogenetic relationships were estimated by neighbor-joining (NJ) (Saitou and Nei 1987) and the maximum parsimony (MP) algorithms using MEGA 5.2 software (Tamura et al. 2011). In addition, a maximum-likelihood (ML) (Rogers and Swofford 1999) analysis was performed using the PhyML program on the Web site http://www.atgc-montpellier.fr/phyml (Guideon and Gascuel 2003). The MODELTEST 3.06 software (Darriba et al. 2012) was used to select the appropriate model of sequence evolution by the Akaike information criterion (AIC), and the GTR + I + G model (α = 0.5870 for the gamma distribution and *p*-inv = 0.6480) was chosen. The robustness of the nodes was evaluated with bootstrap analysis using 1000 pseudoreplicates in NJ, MP methods and Shimodaira–Hasegawa (SH)-like test (Guindon et al. 2010). ML tree was visualized with the program MEGA version 5.2 (Tamura et al. 2011). Similarities among sequences were calculated using the MatGAT v.2.01 software (Campanella et al. 2003).

DNA relatedness estimation

Based on the low 16S rRNA similarities with their nearest neighbors, a whitish-yellow pigmented colony coded as NM2E3^T was selected for further analyses, including the estimation of DNA relatedness in comparison with the most related type strains *Brevibacterium jeotgali* JCM 18571^T and *Brevibacterium yomogidense* JCM 17779^T (purchased from Japan Collection of Microorganisms), as well as *Brevibacterium salitolerans* TRM 415^T (offered by Dr. Tongwei Guan, Xihua University, Chengdu, China).

The genomic DNA was extracted following the protocol described by Marmur (1961), and the DNA relatedness between NM2E3^T and the type strains for related *Brevibacterium* species was performed according to protocols described by De Ley et al. (1970) using the Spectrophotometer Lambda 35 equipped with PTP-1 temperature control (PerkinElmer Inc, USA), and DNA G + C composition of the strain NM2E3^T was determined using a thermal denaturation method (Mandel and Marmur 1968).

Morphological, physiological and biochemical characterization

Cell morphology was observed on well-separated colonies grown on TSA at 28 °C for 3 days. Cell motility was assayed by development of turbidity in test tube containing semisolid TSA medium (0.4 % agar), after 2 days of incubation at 28 °C (Cowan and Steel 1965). Cell dimension and appearance were observed by using a Hitachi S-3400 scanning electron microscope (SEM), and the sample was

prepared using the protocol of Jiao et al. (2015). Bacterial growth was tested at 28 °C on LB agar, nutrient agar (NA), *International Streptomyces Project* (ISP) 1 medium (Pridham and Gottlieb 1948), ISP 4 medium (Shirling and Gottlieb 1966), Czapek's agar and ISP 5 medium (Pridham and Lyons 1961). Diffusible pigments of strain NM2E3^T were analysed on TSA, LB agar, NA, ISP 1 medium, ISP 4 medium and Czapek's agar, and growth abilities were tested on LB agar and/or ISP 4 plates at different temperatures (4, 10, 20, 28 and 37 °C); at different pH values (5.0–12.0 with 1 pH unit increments and adjusted with 1 mol/L HCl or 1 mol/L NaOH after autoclaving); and with different salinities (NaCl 0, 1, 3, 5, 7, 10, 15 and 20 %). The plates were incubated during 3, 5, 7, 15 and 21 days at 28 °C, except for temperature testing. Antibiotics resistance for the strain NM2E3^T and reference species was determined on LB agar medium added with (μg mL^{-1}) tetracycline hydrochloride (5), neomycin sulfate (30), kanamycin sulfate (30), streptomycin sulfate (10), nalidixic acid (30), carbenicillin disodium salt (100), chloramphenicol (30), ampicillin disodium salt (10) and gentamicin sulfate (10). Hydrolysis of starch, Tween 20, Tween 80, and urease, growth on Simmons's citrate, production of hydrogen sulfide, reduction of nitrate and nitrite, Voges–Proskaüer test, catalase and oxidase activities were tested using the protocols described by Cowan and Steel (1965). Assimilation of various carbon compounds was tested using the Biolog GP2 MicroPlates system according to the manufacturer's instructions.

Heavy metal resistance assay

The minimum inhibition concentration (MIC), defined as the lowest concentration of heavy metals that completely inhibits the growth of bacteria, was determined in triplicate for the strain NM2E3^T by streaking it on LB medium plates supplemented separately with heavy metals Cu^{2+} (2–40 mM $CuSO_4$), Zn^{2+} (2–22 mM $ZnSO_4$), As^{5+} (3–480 mM NaH_2AsO_4) and As^{3+} (5.8–28.8 mM $NaAsO_2$). In the case of Pb, the medium proposed by Castañeda-Argullo (1956) was used and the Pb at concentrations of 3.12–20 mM [$Pb(NO_3)_2$] was supplied (Luo et al. 2011). Plates were incubated at 28 °C and growth was observed after 4 days of incubation.

Chemotaxonomic analyses

For analysis of fatty acid profile, strain NM2E3^T and the related reference strains were cultured on TSA, except of *B. jeotgali* JCM 18571^T which was cultivated on LB agar. All the strains were incubated at 28 °C for 72 h. Preparation and analysis of fatty esters were performed as described by Sasser (2001) using the Microbial Identification System (MIDI Inc.) with the Microbial Identification software package (Sherlock version 6.0; MIDI database: RTSBA6) (Tighe et al. 2000). To identify menaquinones, freezing dried cell mass was prepared from the 3-day culture of strain NM2E3^T in TSB at 28 °C. The respiratory quinone was isolated, purified and identified from the strain NM2E3^T according to the procedure described by Komagata and Suzuki (1987). The cell-wall peptidoglycan was prepared by using a method described previously (Komagata and Suzuki 1987). Amino acid compositions were determined using an automatic amino acid analyzer (model L-8900; Hitachi) equipped with a Hitachi custom ion exchange resin.

Results and discussion

In the phylogenetic analysis of the 16S rRNA gene sequences (c.a. 1480 nucleotides), the 60 isolates were divided into 25 genomic species belonging to *Arthrobacter, Bacillus, Brevibacterium, Kocuria, Leucobacter, Microbacterium, Micrococcus, Nocardiopsis, Pseudomonas* and *Staphylococcus* (data not shown). Among these isolates, NM2E3^T was clustered within the genus *Brevibacterium* as shown in the simplified NJ phylogenetic tree of 16S rRNA genes (Fig. 1, complete tree for all the *Brevibacterium* species available as Supplementary Fig. S1). This strain exhibited similarities of 97.3 % with *Brevibacterium jeotgali* JCM 18571^T, 96.6 % with *Brevibacterium yomogidense* JCM 17779^T and 96.1 % with *Brevibacterium salitolerans*

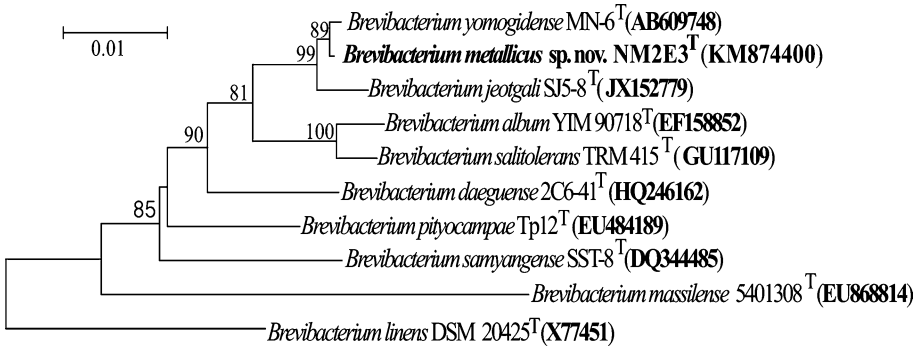

Fig. 1 Simplified 16S RNA neighbor-joining (NJ) tree of *Brevibacterium metallicus* sp. nov. NM2E3^T (in *boldface*) and related species of genus *Brevibacterium*. *Numbers* above branches indicate bootstrap support (>50 %). The *scale bar* presented 1 % substitution of the nucleotides

TRM415^T. This phylogenetic relationship was supported by the MV and MP trees (available as Supplementary Figs. S2 and S3), suggesting that the strain NM2E3^T represents a possible novel species.

The DNA–DNA relatedness between the strain NM2E3^T and *B. yomogidense* JCM 17779^T, *B. jeotgali* JCM 18571^T and *B. salitolerans* TRM 45^T was 39.9, 21.7 and 20.3 %, respectively, which were significantly lower than the threshold (70 %) generally established for the species delineation (Wayne et al. 1987). These results lead to the conclusion that the strain NM2E3^T is a novel genomic species within the genus *Brevibacterium.* The DNA G + C content of the strain NM2E3^T was 70.8 mol % (Tm), within the range of *Brevibacterium* species (62.1–71.7 mol %).

Strain NM2E3^T formed colonies on TSA medium after 48 h with whitish-yellow color, smooth surface, circular shape, convex elevation, and entire edge and diameter of 1.5–3.0 mm. Cells are aerobic, Gram-positive, nonmotile and non-spore-forming rods (0.320–0.429 μm in width and 0.794–1.5 μm in length) (see Supplementary Fig. S4). The differential characteristics of strain NM2E3^T and the related type strains are given in Table 1. Briefly, this strain can be differentiated from the type strains of the related species based on its utilization of D-arabitol, arbutin, D-cellobiose, D-galactose, L-pyroglutamic acid, and its inability to use L-serine, thymine, as well as its resistance to streptomycin at 10 μg mL^{-1}. The results of both the phylogenetic and DNA relatedness analyses make it evident that NM2E3^T represents a novel *Brevibacterium* species.

The MIC values for strain NM2E3^T were 12.5 mM to Pb^{2+}, 40 mM to Cu^{2+}, 72 mM to As^{5+}, 2 mM to Zn^{2+} and 5.8 mM to As^{3+}. Previously, the resistances to arsenic (1 mM) in *Brevibacterium linens* AE038-8 (Maizel et al. 2015), to lead, mercury and cadmium in *Brevibacterium iodium* (De et al. 2008), and to copper (1.57–17.3 mM) in *Brevibacterium antarcticum* (Tashyreva et al. 2009) have been reported, and the latter bacterium could extract 11–75 % of Cu^{2+} from the environment depending on cultivation parameters and copper concentration in the medium. Compared with the results in previous studies, strain NM2E3^T showed much greater resistance to various heavy metals and to arsenic.

Strain NM2E3^T contained MK-8(H_2) (88.4 %) and MK-7(H_2) (11.6 %) as predominant and minor menaquinones for respiration and 2,6-meso-diaminopimelic acid, L-glutamic acid and L-alanine in its peptidoglycan of the cell wall (Table 1), which matched the genus properties (Cai and Collins 1994; DSMZ 2001; Ivanova et al. 2004; Kim et al. 2013). The fatty acid composition of strain NM2E3^T was characterized by predominant amounts of anteiso-$C_{15:0}$ (41.6 %), anteiso-$C_{17:0}$ (30 %) and iso-$C_{15:0}$ (9.5 %). Similar values are also found in the closely related *Brevibacterium* species (Table 2). The presence of 17:1 ω6c (0.6 %) in strain NM2E3^T differentiates it from the closely related species. All of these compounds have been reported as the major fatty acids in the genus *Brevibacterium* (Kim et al. 2013).

Based on these results, we classify the strain NM2E3^T as a novel species within the genus *Brevibacterium* and name it *Brevibacterium metallicus* sp. nov.

Description of *Brevibacterium metallicus* sp. nov

Brevibacterium metallicus (me.tal.li'cus. N. L. masc. adj. *metallicus* referring to the fact that the bacterium is resistant to various heavy metals).

Cells are Gram-positive, aerobic, nonmotile, non-spore-forming rods with size of 0.32–0.43 μm in width and 0.79–1.5 μm in length. Colonies are whitish-yellow, smooth, circular, convex, with entire borders and 1.5–3.0 mm in diameter after 48 h of incubation on TSA medium at 28 °C. On ISP 4 medium, growth temperature range is 20–28 °C with an optimum of 28 °C and growth salinity range is 0–10 %, with optimum of 1 % (w/v). On LB agar medium, the strain grows well at 10–37 °C with optimum of 28–37 °C, at initial pH range 6–12 with 10–11 as optimum, and in the presence of 0–15 % (w/v) NaCl. NM2E3^T grows on LB, TSA, nutritive agar, ISP1, ISP4, ISP 5 and Czapek's agar and produces a brown diffusible pigment in TSA medium after 2 weeks of incubation at 4 °C. It is resistant to (μg/mL) streptomycin (10), nalidixic acid (30) and tetracycline (5) and is susceptible to carbenicillin (100), chloramphenicol, neomycin and kanamycin (30), ampicillin and gentamycin (10). Oxidase and catalase are positive. Nitrate is reduced to nitrite. Starch and Tween 20 are not hydrolyzed. Urease, assimilation of citrate, H_2S production, Vogues–Proskaüer test and nitrite reduction are negative. With the GP2 system, the strain NM2E3^T is able to assimilate β-cyclodextrin, Tween 40, Tween 80, L-arabinose, D-arabitol, arbutin, D-cellobiose, D-galactose, ribose, D-xylose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, lactamide, L-lactic acid, L-malic acid, L-pyruvic acid methyl ester, succinic acid monomethyl ester, propionic acid, succinamic acid, L-pyroglutamic acid, L-asparagine, L-alanylglycine, L-alanine, L-alaninamide, pyruvic acid, succinic acid, propionic acid and 2,3-butanodiol, whereas assimilation of gentibiose, α-D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-melezitose, D-melobiose, β-methyl-D-glucose, α-methyl-D-mannoside, palatinose, D-psicose, D-raffinose, L-rhamnose, D-trehalose, ρ-hydroxyphenylacetic acid, putrisine, uridine, uridine-5′monophosphate, α-D-glucose-1-phosphate and D,L-α-glycerolphosphate is negative. In addition, dextrin, glycogen, D-fructose, α-D-glucose, m-inositol, D-mannose, 3-methyl-D-glucose, salicin, D-sorbitol, D-tagatose, xylitol,

Table 1 Differential phenotypic features of strain NM2E3^T and closely related members of the genus *Brevibacterium*

Characteristics	Tested strains			
	1	2	3	4
Morphology	Rods	Short rods	Rods	Rods/cocci
Colony color	Whitish-yellow	Whitish-yellow	Yellow	Pale-yellow
Oxidase	+	−	+	−
Growth in/at				
4 °C	−	w	−	w
37 °C	+	+	−	+
NaCl 15 %	w	+	w	−
pH 6	+	+	w	+
pH11	+	+	w	+
ISP 4	+	+	w	w
ISP 5	+	+	−	w
Czapeck	+	+	w	w
Antibiotic resistance ($\mu g/mL^{-1}$)				
Streptomycin (10)	+	−	−	−
Nalidixic acid (30)	+	+	−	+
Ampicillin (10)	+	+	−	−
Tetracycline (5)	+	+	−	+
Kanamycin (10)	−	w	−	−
Carbenicillin (100)	−	w	−	−
Utilization of carbon source in Biolog GP2 microplates				
α-Cyclodextrin	−	w	−	−
β-Cyclodextrin	+	+	+	−
Dextrin	w	+	−	+
Glycogen	w	+	−	−
Inulin	−	+	−	−
Mannan	−	+	−	−
N-acetyl D-glucosamide	−	+	−	−
N-acetyl D-mannosamine	−	+	−	−
Amygdalin	−	+	−	−
L-Arabinose	+	w	+	+
D-Arabitol	+	−	−	−
Arbutin	+	−	−	−
D-Cellobiose	+	−	−	−
D-Fructose	w	−	−	−
L-Fucose	w	w	−	−
D-Galactose	+	−	−	w
D-Galacturonic acid	−	−	−	w
D-Gluconic acid	−	+	−	−
α-D-Glucose	w	+	−	w
m-Inositol	w	+	−	−
D-Mannose	w	w	−	w
β-Methyl-D-glucoside	−	+	−	−
3-Methyl-D-glucose	w	+	−	w
Salicin	w	−	−	−
Sedoheptulosan	−	w	−	−
D-Sorbitol	w	+	w	−
Stachyose	−	+	−	−
Sucrose	−	+	−	−

Table 1 continued

Characteristics	Tested strains			
	1	2	3	4
D-Tagose	w	−	+	w
Turanose	w	−	−	−
Xylitol	w	−	w	−
γ-Hydroxybutyric acid	w	+	+	+
Lactamide	+	−	+	+
D-Lactic acid methylester	w	+	−	+
D-Malic acid	−	−	+	w
Pyruvic acid methylester	+	+	+	−
Succinamic Acid	+	w	+	+
Succinic Acid	+	+	−	w
L-Alaninamide	+	w	+	+
L-Glutamic acid	−	+	−	w
Glycyl L-glutamic acid	−	+	−	−
L-Pyroglutamic acid	+	−	−	−
L-Serine	−	+	+	+
2,3 Butanediol	+	−	w	−
Glycerol	w	w	+	+
Adenosine	w	−	w	w
2-Deoxyadenosine	w	+	−	−
Inosine	w	−	−	−
Thymidine	−	+	+	w
Adenosine 5´monophosphate	w	−	−	−
Thymidine 5´monophosphate	−	w	−	w
D-Fructose phosphate	−	−	+	−
Quinones	MK-8(H_2) MK-7(H_2)	MK-6(H_2) MK-7(H_2) MK-8(H_2) MK-6,MK-7 MK-8	MK-8(H_2)	MK-7(H_2) MK-8(H_2)
Cell-wall sugar	NA	Gal, Xyl,Rib	NA	Gal,Xyl
DNA G +C content (mol %)	70.8	69	69.3	67.4

Strains: 1, *Brevibaterium metallicus* sp. nov. NM2E3^T; 2, *Brevibacterium salitolerans* TRM 415^T; 3, *Brevibacterium jeotgali* JCM 18571^T; 4, *Brevibacterium yomogidense* JCM 17779^T. All strains are positive for the following characteristics: Gram stain; growth in LB medium with 0–10 % (w/v) of NaCl and in ISP 4 medium with 0–7 % (w/v) of NaCl; growth in LB agar, TSA and ISP1; catalase; metabolism of Tween 40, Tween 80, Ribose, D-xylose, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, L-lactic acid, L-malic acid, succinic acid monomethyl ester, propionic acid, pyruvic acid, L- alanine, L-alanylglycine and L-asparagine. All strains are negative for growth at 4–10 °C in ISP 4 medium and at pH 5; resistance to chloramphenicol (30 μg/mL), neomycin (30 μg/mL) and gentamycin (30 μg/mL); no motility; hydrolysis of starch and urea; assimilation of gentibiose, α-D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-melezitose, D-melobiose, β-methyl-D-glucose, α-methyl-D-mannoside, palatinose, D-psicose, D-raffinose, L-rhamnose, D-trehalose, ρ-hydroxyphenylacetic acid, putrisine, uridine, uridine-5′monophosphate, α-D-glucose-1-phosphate and D,L-α-glycerolphosphate. +, positive; −, negative, *w* weakly positive, *NA* not available

γ-hydroxybutyric acid, D-lactic acid methyl ester, *N*-acetyl-L-glutamic acid, D-alanine, glycerol, adenosine, 2´-deoxy-adenosine, inosine and adenosine 5´-phosphate are also utilized by the test strain, but the purple color was only developed slightly compared with the compounds mentioned above.

The major fatty methyl esters are anteiso-$C_{15:0}$ (41.6 %), anteiso-$C_{17:0}$ (30 %) and iso-$C_{15:0}$ (9.5 %). The major respiratory quinone is MK-8 (H2) and a minor amount of MK-7 (H2) is also present. Cell-wall diamino acids are 2,6-meso-diaminopimelic acid, L-glutamic acid and L-alanine. The strain NM2E3^T presents 70.8 mol % (Tm) of

Table 2 Cellular fatty acid content (%) of strain NM2E3^T (*Brevibacterium metallicus* sp. nov.) and related type strains of the genus *Brevibacterium*

Fatty acids	1	2	3	4
Saturated fatty acid				
14:00	TR	TR	0.75	0.96
16:00	4.5	3.5	**8.8**	**10.7**
18:00	1.6	1.2	3.9	3.2
Branched				
14:0 iso	0.5	TR	0.7	–
15:0 iso	**9.5**	**11.8**	**13.0**	**8.4**
16:0 iso	4.5	4.3	3.9	2.2
16:0 iso G	TR	–	TR	–
17:0 iso	1.8	2.2	2.6	1.0
13:0 anteiso	TR	–	–	–
15:0 anteiso	**41.9**	**43.6**	**34.0**	**47.5**
15:1 ω5c	–	–	TR	–
16:0 anteiso	–	TR	1.0	–
17:0 anteiso	**30**	**32**	**24.7**	**24.4**
17:0 anteiso A	1.0	–	1.1	0.6
17:1 ω8c	0.7	–	1.9	–
17:1 ω6c	0.6	–	–	–
17:1 ω7c	–	0.5	–	–
18:1 ω9c	TR	TR	0.5	0.5
Summed features$^+$				
7	0.5	–	TR	–
8	0.8	TR	0.5	TR

Taxa: 1, strain NM2E3^T ; 2, *Brevibacterium salitolerans* TRM 415^T; 3, *Brevibacterium jeotgali* JCM 18571^T; 4, *Brevibacterium yomogidense* JCM 17779^T. All the data presented were obtained from this study. Data are shown in percentages of total fatty acids. Major components (≥5 %) in bold type. Strains containing less than 0.5 % of fatty acids are shown as "TR"; "-" represents not detected. Summed features represent groups of two or three fatty acids that could not be separated by GLC with MIDI system. Summed feature 7 contained 19:1 ω7c/19:1 ω6c. Summed feature 8 contained 18:1 ω7c

DNA G + C content. The type strain NM2E3^T (=CCBAU 101093^T = HAMBI 3627^T = LMG 8673^T) was isolated from the root of *Prosopis laegivata* grown at the edge of a mine tailing in Villa de la Paz in the state of San Luis Potosi (23.7 N, 178.7 W) in Mexico.

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Compliance with ethical standards

Conflict of interest The authors declare they have no competing interests and they were notified about the content of the manuscript.

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