


A novel pigmented and heavy metal biosorptive bacterium, *Leucobacter epilobiisoli* sp. nov., isolated from rhizosphere soil of *Epilobium hirsutum* L.

Shao-Yang Hou · Fang Wang · Meng-Yue Zhang · Juan Cheng ·
Yi-Xuan Zhang 

Received: 6 February 2018 / Accepted: 20 March 2018 / Published online: 22 March 2018
© Springer International Publishing AG, part of Springer Nature 2018

Abstract A novel yellow pigmented, Gram-positive, aerobic and heavy metal biosorptive bacterium designated SYP-B2667^T was isolated from rhizosphere soil of *Epilobium hirsutum* L. in Tongren, Guizhou province, China. Based on 16S rRNA gene sequence analyses, it was shown that strain SYP-B2667^T represents a novel species in the genus *Leucobacter*, with *Leucobacter chromiireducens* subsp. *solipictus* JCM 15573^T as a close phylogenetic neighbour (sequence similarity of 98.2%). Chemotaxonomic characteristics also supported the affiliation to the genus *Leucobacter*. Strain SYP-B2667^T was determined to have a DNA G+C content of 66.6 mol%; 2,4-diaminobutyric acid in the cell wall peptidoglycan amino acids; MK-11 as predominant menaquinone; an abundance of anteiso-C_{15:0} and anteiso-C_{17:0} fatty acids; and polar lipids including diphosphatidylglycerol, phosphatidylglycerol, glycolipids and unidentified phospholipids. The DNA–DNA hybridization value between strain SYP-B2667^T and

L. chromiireducens subsp. *solipictus* JCM 15573^T was 19.7 ± 2.8%. Based on these phylogenetic and phenotypic results, it can be concluded that strain SYP-B2667^T represents a novel species, for which the name *Leucobacter epilobiisoli* sp. nov. is proposed. The type strain is SYP-B2667^T (=DSM 105145^T=CPCC 204976^T). This strain can tolerate and adsorb five heavy metals and so may have potential to facilitate heavy metal removal and bioremediation.

Keywords *Epilobium hirsutum* L. · Heavy metals biosorption · *Leucobacter* sp. nov. · Rhizosphere soil

Introduction

Epilobium hirsutum L. (*Onagraceae* family), commonly known as great willowherb, is a perennial, flowering plant widespread in most of Europe, North Africa and parts of Asia, typically growing in wet habitats. *E. hirsutum* L. is notable for its content of ellagitannins and flavonoids (Toth et al. 2008) and its extracts have high-efficiency antimicrobial activity (Lucia et al. 2015). It is also an endangered plant and easily affected by substances in the soil (Lee et al. 2017), especially heavy metals which are harmful to the growth of plants (Ou et al. 2016). Thus, reducing heavy metals in soil is necessary.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10482-018-1071-3>) contains supplementary material, which is available to authorized users.

S.-Y. Hou · F. Wang · M.-Y. Zhang ·
J. Cheng · Y.-X. Zhang (✉)
School of Life Science and Biopharmaceutics, Shenyang
Pharmaceutical University, Shenyang 110016, People's
Republic of China
e-mail: zhangyxzsh@163.com

In the present exploration of microbial diversity in rhizosphere soil of *E. hirsutum* L., a strain in genus *Leucobacter* with high heavy metal tolerance and biosorption activities was found. The genus *Leucobacter* was first proposed by Takeuchi et al. (1996) to allow taxonomic differentiation of distinct phylogenetic lineages within the family *Microbacteriaceae*. The genus *Leucobacter* was proposed to accommodate Gram-positive rods with 2,4-diaminobutyric acid (DAB) in their cells and a high percentage of menaquinone-11 (MK-11) (Takeuchi et al. 1996). At the time of writing, the genus *Leucobacter* comprises 23 species and 6 subspecies. Species of genus *Leucobacter* have been isolated from various ecological niches, such as *Leucobacter weissii* from activated sludge (Schumann and Pukall 2017), *Leucobacter komagatae* from an ampoule contaminant (Takeuchi et al. 1996), *Leucobacter margaritifformis* from bamboo extract (Lee and Lee 2012), *Leucobacter corticis* and *Leucobacter populi* from bark (Fang et al. 2016; Li et al. 2016), *Leucobacter ruminantium* from bovine rumen (Chun et al. 2017), *Leucobacter chironomi* from a chironomid egg mass (Halpern et al. 2009), *Leucobacter chromiireducens*, *Leucobacter aridicollis* and *L. chromiireducens* subsp. *chromiireducens* from chromium contaminated environments (Morais et al. 2004, 2005), *Leucobacter exalbidus* from compost (Harumi 2011), *Leucobacter denitrificans* from cow dung (Weon et al. 2012), *Leucobacter aerolatus* from duck barn air (Martin et al. 2010), '*Leucobacter kyeonggiensis*' from dye waste water (Kim and Lee 2011), *Leucobacter celer* and *L. celer* subsp. *celer* from fermented seafood (Shin et al. 2011; Clark and Hodgkin 2015), *Leucobacter holotrichiae* from larval gut of *Holotrichia oblita* (Zhu et al. 2016), *Leucobacter zaeae* from maize rhizosphere (Lai et al. 2015), *L. chromiireducens* subsp. *solipictus*, *Leucobacter musarum* subsp. *musarum*, *L. musarum* subsp. *japonicus* and *L. celer* subsp. *astrifaciens* from nematode (Muir and Tan 2007; Clark and Hodgkin 2015), *Leucobacter tardus* from phyllosphere (Behrendt et al. 2008), *Leucobacter luti* and *Leucobacter alluvii* from river sediment (Morais et al. 2006), *Leucobacter salsicius* from salt-fermented food (Yun et al. 2011), *Leucobacter triazinivorans* from sludge (Sun et al. 2017), *L. chromiireducens* and *Leucobacter albus* from soil (Sturm et al. 2011; Lin et al. 2004). *Leucobacter* spp. are found in metal stressed communities and a few chromate tolerant strains of this genus

have been reported from chromium contaminated sludge and sediments, such as *L. chromiireducens* CRB2^T with Cr(VI) reduction potential, which may be useful for the growth of plants and environmental decontamination (Sarangi and Krishnan 2008; Joutey et al. 2016).

Isolation of bacteria from soil would represent an appropriate practice to select metal resistant strains that could be used for heavy metal removal and bioremediation purposes (Morais et al. 2004). In this work, strain SYP-B2667^T, isolated from rhizosphere soil of *E. hirsutum* L., was found to have high 16S rRNA gene sequence similarity with *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T (98.2 and 97.6%, respectively), but to have distinctive phenotypic and phylogenetic characteristics. Moreover, strain SYP-B2667^T has the ability to tolerate and adsorb five heavy metals. Thus, further studies were performed to determine the taxonomic position of strain SYP-B2667^T and its potential to reduce the heavy metal contamination.

Materials and methods

Bacterial strains and culture conditions

Strain SYP-B2667^T was isolated from rhizosphere soil of *E. hirsutum* L. collected from Tongren, Guizhou province, China (46°16'23"N, 123°16'22"W). Using the method of gradient dilution coating (Williams and Crawford 1983), the diluted soil suspension was incubated on Tryptic Soy Agar (Difco) supplemented with 100 mg l⁻¹ nystatin at 28 °C for 2 days colonies were picked up and re-streaked repeatedly onto Luria–Bertani (LB) agar (Oxoid) until the purity was confirmed. The purified strain was preserved in glycerol suspensions (30.0%, v/v) at – 80 °C.

The type strains of *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T were obtained from the Japan Collection of Microorganisms (JCM) and cultured under comparable conditions for reference purposes (Muir and Tan 2007).

Phylogenetic analysis

The 16S rRNA gene sequencing of strain SYP-B2667^T was performed according to the method reported by Cui et al. (2001) and Li et al. (2007). The obtained sequence was compared with the available 16S rRNA gene sequences of validly name species in the EzBioCloud server (<http://www.ezbiocloud.net>) (Yoon et al. 2017). Phylogenetic trees were reconstructed with three algorithms, neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) using the software package MEGA version 7.0 (Sudhir et al. 2016) after multiple alignment of the sequence data by the CLUSTAL X program (Thompson et al. 1997). Pairwise distances for the neighbor-joining algorithm (Saitou and Nei 1987) were calculated according to Kimura two-parameter model (Kimura 1980). Bootstrap analysis was performed with 1000 replications (Felsenstein 1985).

Morphological, physiological and biochemical tests

Cells were grown in either liquid or on solid (2.0% agar) LB medium under aerobic conditions at 28 °C, unless otherwise indicated. Cell morphology and motility were determined by using staining transmission electron microscopy (HT 7700; Hitachi, Japan). Gram-staining was carried out using the standard Gram-reaction (Claus 1992). The temperature range for growth was determined by incubating slant cultures (LB medium, pH 7.2) from 0 to 45 °C. The pH range for growth was determined in liquid medium that was adjusted to pH 5.0 using citric acid and sodium citrate, pH 6.0, 7.0 and 8.0 with NaOH and KH₂PO₄, pH 9.0 and 10.0 with Na₂CO₃ and NaHCO₃, pH 11.0 with Na₂HPO₄ and NaOH, and pH 12.0 and 13.0 with KCl and NaOH (Xu et al. 2005). Halotolerance was examined in solid cultures containing NaCl at concentrations of 0–10.0% (w/v; with an interval of 1.0%). Tolerance of strain SYP-B2667^T against different concentrations of streptomycin, tetracycline, chloromycetin, kanamycin on LB-agar plates was evaluated until the strain was unable to produce colonies on the agar plates. Oxidase activity was determined by the oxidation of tetramethyl-*p*-phenylenediamine (Kovacs 1956). Catalase activity was detected by assessing the production of bubbles

on addition of a drop of 3.0% (v/v) H₂O₂. H₂S production and decomposition of cellulose, starch, Tweens 20, 40, 60 and 80 were performed as described by Gonzalez et al. (1978). Carbon source utilisation and nitrogen source utilisation were determined according to the methods of Shirling and Gottlieb (1966).

The isoprenoid quinones were extracted and analysed using HPLC (Collins et al. 1977; Kroppenstedt 1982). Sugar analysis of whole cell hydrolysates were performed according to the procedures described by Tang et al. (2009). Purified cell wall preparations were obtained and hydrolysed as described by Schleifer and Kandler (1972) and amino acids in cell wall peptidoglycan hydrolysates were analysed using an automatic amino acid analyzer (L-8900, Hitachi, Japan). The method of Schleifer and Kandler (1972) was also utilised with a modification of thin layer chromatography on cellulose sheets instead of paper chromatography. Cellular fatty acid analysis was performed by using the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6 (Sasser 1990)). Polar lipids of strain SYP-B2667^T were extracted as described by Minnikin et al. (1979) and identified by two-dimensional thin layer chromatography (Collins and Jones 1980). DNA–DNA hybridization was performed fluorometrically, according to the method described by Ezaki et al. (1989). The DNA G+C content was determined by reversed-phase HPLC using *Escherichia coli* DH5 α as the reference strain, as described by Mesbah et al. (1989). All tests were carried out simultaneously with strain SYP-B2667^T and the two type strains *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T.

Evaluation of heavy metals tolerance and biosorption

Tolerance of strain SYP-B2667^T to different concentrations of As(V), Cd(II), Cr(VI), Cu(II) and Pb(II) on LB-agar plates was evaluated until the strain was unable to produce colonies. The heavy metal biosorption ability of strain SYP-B2667^T was determined through cultivation in LB-liquid medium. 100 μ l of cells ($\sim 10^7$ cfu ml⁻¹) were inoculated into 45 ml LB broth medium and incubated at 28 °C at 200 rpm, treated with 60 mg l⁻¹ of As(V), Cd(II), Cr(VI), Cu(II) and Pb(II), respectively. LB liquid medium

without strain SYP-B2667^T, treated with equal concentrations of heavy metals was used as control group. At cultivation intervals of 24, 48 and 72 h, the cultures in the treatment and control groups were simultaneously harvested by centrifugation (Joutey et al. 2016). Treatments of biomass followed the method of Li et al. (2017). The fermentation broth was treated with an equal volume of 6.0% nitric acid. Concentrations of heavy metals in biomass and fermentation broths were measured by ICP-MS (ICAP Q, Thermo, USA) (Rui et al. 2008). Disodium hydrogen arsenate (Na₂AsO₄·7H₂O), cadmium chloride hemi (CdCl₂), potassium dichromate (K₂Cr₂O₇), cupric chloride (CuCl₂) and lead dinitrate (PbN₂O₆) were used as the sources of As(V), Cd(II), Cr(VI), Cu(II) and Pb(II), and purchased from National Standard Substances Center (Beijing, China).

Results and discussion

Phylogenetic analysis

The 16S rRNA gene sequence of strain SYP-B2667^T was found to be a continuous stretch of 1531 bp (GenBank accession number MF179851). The Blast analysis of the 16S rRNA gene sequence at the EzBioCloud server showed high similarity to *L. chromiireducens* subsp. *solipictus* JCM 15573^T (98.2%) and *L. chromiireducens* JCM 13322^T (97.6%). Other current *Leucobacter* species showed 16S rRNA gene similarities to strain SYP-B2667^T of 94.2–96.4%. In the phylogenetic tree based on the neighbour-joining algorithm, strain SYP-B2667^T formed a monophyletic clade with *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T (Fig. 1).

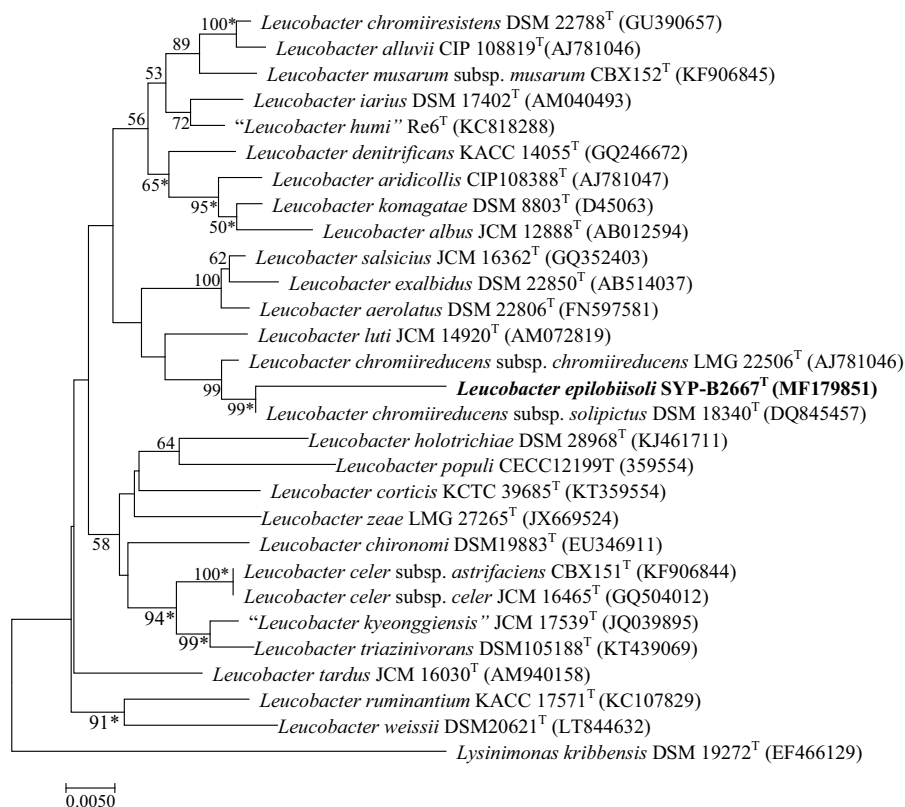


Fig. 1 Neighbor-joining phylogenetic tree based on 16S ribosomal RNA gene sequences, showing the position of strain SYP-B2667^T. Bootstrap values are shown as percentages of 1000 replicates, with those above 50% shown at the branch points. *Lysinimonas kribbensis* DSM19272^T (EF466129) was

used as an outgroup. Asterisks denote nodes that were also recovered using the maximum-parsimony and maximum likelihood methods. Bar, 0.005 substitutions per nucleotide position

Table 1 Differential characteristics between strain SYP-B2667^T and related reference type strains

	SYP-B2667 ^T	JCM15573 ^T	JCM13322 ^T
Colony colour	Yellow	Yellow	Cream
DNA G+C content (mol%)	66.6	69.5 ^a	66.7 ^b
Temperature range for growth (°C)	7.0–37.0	5.0–34.0	2.0–37.0
Optimum (°C)	28.0	30.0	30.0
pH range	6.0–10.0	6.0–11.0	6.0–10.0
Optimum	6.0	8.0	7.0
Antibiotics tolerant concentration			
Streptomycin (µg l ⁻¹)	40.0	300.0	50.0
Tetracycline (µg l ⁻¹)	30.0	12.5	12.5
Chloromycetin (µg l ⁻¹)	150.0	20.0	20.0
Kanamycin (µg l ⁻¹)	1.0	50.0	50.0
NaCl range (w/v, %)	0–5.0	0–6.0	0–5.0
Optimum (w/v, %)	0	1.0	1.0
Assimilation of			
L-Alanine	–	+	+
L-Methionine	–	+	+
L-Lysine	+	+	–
L-Phenylalanine	–	+	–
L-Proline	w	+	+
L-Valine	–	+	+
D-Fructose	–	w	–
D-Galactose	w	+	+
D-Glucose	w	+	–
D-Lyxose	–	+	+
D-Xylose	+	+	–
Enzyme activity			
Catalase	w	+	w
Urease	–	+	–
Hydrolysis of			
Milk coagulation and peptonization	+	+	–
Starch	–	+	+
Tween 20	+	w	w
Tween 40	+	–	+
Tween 80	w	+	+
Major polar lipids	DPG, PG, GL	DPG, PG ^a	DPG, PG

Data for reference strains are from this study unless indicated

DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *GL* glycolipids, *PL* unidentified phospholipid, “+” positive, “–” negative, *w* weakly positive

^aData obtained from Muir and Tan (2007)

^bData obtained from Yun et al. (2011)

Topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms (Figs. S1, S2) were similar to that of the tree constructed by neighbour-joining analysis.

Morphological, physiological and biochemical tests

Strain SYP-B2667^T was observed to be an aerobic, Gram-positive, non-motile and rod-shaped bacterium

(Fig. S3). Strain SYP-B2667^T yields pale yellow-coloured circular, convex colonies, 0.5–0.8 µm in width and 1.0–2.0 µm in length when cultured on LB agar at 28.0 °C. Strain SYP-B2667^T was found to grow at 4.0–37.0 °C, pH 6.0–10.0 and in the presence of 0–5.0% (w/v) NaCl. Optimum growth was observed at 28 °C, pH 6.0 without NaCl. The maximum concentrations of various antibiotics tolerated are indicated in Table 1. The physiological characteristics of strain SYP-B2667^T are summarised in the species

Table 2 Major fatty acids (> 0.5%) of SYP-B2667^T and related reference type strains

Fatty acid	SYP-B2667 ^T	JCM 15573 ^T	JCM 13322 ^T
anteiso-C15:0	52.5	55.6	47.8
anteiso-C17:0	26.3	20.5	26.2
iso-C14:0	TR	TR	1.2
iso-C15:0	0.8	1.1	3.3
iso-C16:0	15.0	11.1	17.5
iso-C17:0	TR	TR	0.7
iso-C15:1	–	TR	–
C14:0	TR	TR	TR
C15:0	TR	–	–
C16:0	3.6	10.4	2.9
C17:0	TR	–	–
C18:0	–	TR	–
C16:1 ω 7c	TR	–	–
C18:1 ω 9c	TR	–	–

Data are from the present study. All strains were grown on Tryptic Soy Agar at 28.0 °C for 3 or 4 days until sufficient biomass could be obtained

“–” not detected, TR trace (< 0.5%)

description and a comparison of selective characteristics with related type strains are shown in Table 1.

The predominant quinone of strain SYP-B2667^T was found to be MK-11 (77%) which is similar to all other members of the genus *Leucobacter*. A minor amount of MK-10 (23%) was also observed. The whole cell hydrolysate of strain SYP-B2667^T was found to contain galactose, glucose, mannose, rhamnose and ribose. The cell wall peptidoglycan was found to contain DAB, alanine, glycine and glutamic acid. The cellular fatty acids of strain SYP-B2667^T were found to include major amounts of anteiso-C_{15:0} (52.5%), anteiso-C_{17:0} (26.3%), iso-C_{17:0} (15.0%) and C_{16:0} (3.6%). The cellular fatty acid profiles of strain

SYP-B2667^T and related *Leucobacter* type strains are shown in Table 2. The polar lipid components of strain SYP-B2667^T were identified as diphosphatidylglycerol, phosphatidylglycerol, glycolipids and unidentified phospholipids (Fig. S4). Similar lipids were also reported to occur in other *Leucobacter* species (Lee and Lee 2012; Muir and Tan 2007; Yun et al. 2011).

Strain SYP-B2667^T and related species show the same general characteristics: non-motile, catalase positive, rod-shaped cells; the presence of DAB, alanine, glycine and glutamic acid cell wall peptidoglycan; anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{16:0} as dominant fatty acids; and MK-11 as the major menaquinone. A detailed comparison of the peptidoglycan composition of all described *Leucobacter* species is given in Table S1. All species in the genus *Leucobacter* have DAB, 22 species and 6 subspecies have alanine, glycine and glutamic acid, as in strain SYP-B2667^T, which does not have gamma-aminobutyric acid, lysine or threonine in the peptidoglycan.

Muir and Tan (2007) described two *L. chromiireducens* subspecies, *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T. The two subspecies exhibit similar metabolic profiles, share 99.5% 16S rRNA gene sequence similarity and their mean DNA–DNA relatedness was approximately 91%. Differences between two subspecies include colony colour, ability to reduce hexavalent chromium and the rate of producing moderately hydrophobic lawns. SYP-B2667^T exhibited many different characteristics (Tables 1, 2) and the 16S rRNA sequence similarities of strain SYP-B2667^T with *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T are 98.2 and 97.6%; DNA–DNA relatedness with *L. chromiireducens* subsp. *solipictus* JCM 15573^T and *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T were 19.7 ± 2.8 and 15.9 ± 2.3%. Despite the 16S rRNA gene

Table 3 ICP-MS detection of heavy metal biosorption (24, 48, 72 h) by strain SYP-B2667^T (mean value ± SD; n = 3)

Heavy metals	Biosorption (%)			Medium residual level (%)		
	24 h	48 h	72 h	24 h	48 h	72 h
As(V)	7.5 ± 1.3	36.8 ± 2.3	56.7 ± 3.5	91.6 ± 2.4	62.1 ± 2.1	42.1 ± 1.0
Cd(II)	8.6 ± 0.8	48.7 ± 3.5	65.8 ± 2.3	89.6 ± 3.5	50.3 ± 1.6	32.5 ± 1.6
Cr(VI)	10.2 ± 1.2	57.6 ± 1.5	71.9 ± 4.8	86.9 ± 2.8	40.5 ± 1.9	26.7 ± 1.6
Cu(II)	5.6 ± 0.8	25.6 ± 2.1	43.2 ± 1.2	93.6 ± 3.2	73.2 ± 3.9	53.6 ± 3.2
Pb(II)	9.5 ± 1.1	56.8 ± 3.4	61.3 ± 2.4	89.6 ± 1.9	41.6 ± 3.0	35.6 ± 1.2

sequence analyses placing strain SYP-B2667^T with *L. chromiireducens* subspecies (Figs. 1, S1, S2) it is clear that there are substantial differences between SYP-B2667^T and these two subspecies. The G+C content of strain SYP-B2667^T was found to be 66.6 mol%, which is distinct from *L. chromiireducens* subsp. *solipictus* JCM 15573^T (69.5 mol%), although similar to *L. chromiireducens* subsp. *chromiireducens* JCM 13322^T (66.7 mol%).

On the basis of phenotypic, chemotaxonomic and phylogenetic analyses, SYP-B2667^T is concluded to represent a novel species of the genus *Leucobacter*, for which the name *Leucobacter epilobiisoli* sp. nov. is proposed. The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for strain SYP-B2667^T is TA00365.

Heavy metal tolerance and biosorption ability

Strain SYP-B2667^T showed tolerance against 350.0 mg l⁻¹ As(V), 450.0 mg l⁻¹ Cd(II), 700.0 mg l⁻¹ Cr(VI), 400.0 mg l⁻¹ Cu(II) and 300.0 mg l⁻¹ Pb(II). The heavy metal biosorption levels increased over 24, 48 and 72 h, and the residual levels in the medium gradually declined (Table 3). The biosorption level at 72 h were all above 40%, which demonstrated that strain SYP-B2667^T has considerable potential for biosorption of heavy metals.

A few Cr(VI) tolerant strains of the genus *Leucobacter* have been isolated from chromium contaminated sludge and sediments (Sarangi and Krishnan 2008; Joutey et al. 2016). All these strains could tolerate Cr(VI) below 700 mg l⁻¹, lower than that of strain SYP-B2667^T; no other heavy metal biosorption has been reported for members of the genus *Leucobacter*, so it is significant that strain SYP-B2667^T has a notable ability to absorb heavy metals; to our knowledge this is the first report of a *Leucobacter* species that can tolerate five heavy metals. The strain thus has potential for resisting and remediating pollution by multiple heavy metals, which is highly desirable for bioremediation processes.

Description of *Leucobacter epilobiisoli* sp. nov.

Leucobacter epilobiisoli (e.pi.lo.bi.i.so'li. N. L. neut. n. *epilobium* willowherb; L. neut. n. *solum* soil; N.L. gen. n. *epilobiisoli* of soil of an *Epilobium* root).

Aerobic, yellow coloured, Gram-stain positive, non-motile (on semi-solid agar medium), rods of 0.5–0.8 µm width and 1.0–2.0 µm length. Optimum growth is at 28 °C, pH 6.0 and in the presence of 0% (w/v) NaCl. Catalase positive and oxidase negative. Hydrolysis of cellulose, gelatin and starch are negative. Hydrolysis of Tweens (20, 40, 60 and 80) is positive. Positive for indole production from tryptophan, milk coagulation and peptonisation. Negative for urease, H₂S production, nitrate reduction, methyl red test and Voges–Proskauer test. Assimilates histidine and proline but not alanine, glutamic, lysine, methionine, phenylalanine or valine. Assimilates fucose, galactose, glucose, mannose, raffinose and xylose but not arabinose, fructose, lyxose or rhamnose. The amino acids of the cell wall peptidoglycan are DAB, alanine, glycine and glutamic acid. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, glycolipids and unidentified phospholipids. The major fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and C_{16:0}. The G+C content of the type strain is 66.6 mol%.

The type strain is SYP-B2667^T (=DSM 105145^T=CPCC 204976^T), isolated from the rhizosphere soil of *E. hirsutum* L. in Tongren, Guizhou Province, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence is MF179851.

Acknowledgements This research was supported by the Major Science and Technique Programs in Yunnan Province (2016ZF001-001, 2017IB038), Project of Yunnan provincial academy of science and technology (2015IC017) and National Science and Technology Major Project (2018ZX09735001-002-002).

Compliance with ethical standards

Conflict of interest The authors declare that they have no direct or indirect conflict of interest.

Ethical approval This is the original work of the authors. The work described has not been submitted elsewhere for publication, in whole or in part, and all authors listed carried out the data analysis and manuscript writing. Moreover, all authors read and approved the final manuscript.

References

- Behrendt U, Ulrich A, Schumann P (2008) *Leucobacter tardus* sp. nov., isolated from the phyllosphere of *Solanum tuberosum* L. Int J Syst Evol Microbiol 58:2574–2578

- Chun BH, Lee HJ, Jeong SE, Schumann P, Jeon CO (2017) *Leucobacter ruminantium* sp. nov., isolated from the bovine rumen. *Int J Syst Evol Microbiol* 67:2634–2639
- Clark LC, Hodgkin J (2015) *Leucobacter musarum* subsp. *musarum* sp. nov., subsp. nov., *Leucobacter musarum* subsp. *japonicus* subsp. nov., and *Leucobacter celer* subsp. *astrifaciens* subsp. nov., three nematopathogenic bacteria isolated from *Caenorhabditis*, with an emended description of *Leucobacter celer*. *Int J Syst Evol Microbiol* 65:3977–3984
- Claus D (1992) A standardized gram staining procedure. *World J Microbiol Biotechnol* 8:451–452
- Collins MD, Jones D (1980) Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Microbiol* 63:45–470
- Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 100:221–230
- Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardioseae*. *Int J Syst Evol Microbiol* 51:357–363
- Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Evol Microbiol* 39:224–229
- Fang W, Li Y, Xie SJ, Yang X, Wang LF (2016) *Leucobacter populi* sp. nov., isolated from symptomatic bark of *Populus × euramericana* canker. *Int J Syst Evol Microbiol* 66:2254–2258
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20:406–416
- Gonzalez C, Gutierrez C, Ramirez C (1978) *Halobacterium vallismortis* sp. nov. an amyolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* 24:710–715
- Halpern M, Shaked T, Pukall R, Schumann P (2009) *Leucobacter chironomi* sp. nov., a chromate-resistant bacterium isolated from a chironomid egg mass. *Int J Syst Evol Microbiol* 59:665–670
- Harumi U (2011) *Leucobacter exalbidus* sp. nov., an actinobacterium isolated from a mixed culture from compost. *J Gen Appl Microbiol* 57:27–33
- Joutey NT, Bahafid W, Sayel H, Nassef S, Ghachtouli NE (2016) *Leucobacter chromiireducens* CRB2, a new strain with high Cr(VI) reduction potential isolated from tannery-contaminated soil (Fez, Morocco). *Ann Microbiol* 66:425–436
- Kim H, Lee S (2011) *Leucobacter kyeonggiensis* sp. nov., a new species isolated from dye waste water. *J Microbiol* 49:1044–1049
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 4535:703–704
- Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* 12:2359–2367
- Lai WA, Lin SY, Hameed A, Hsu YH, Liu YC, Huang HR, Shen FT, Young CC (2015) *Leucobacter zae* sp. nov., isolated from the rhizosphere of maize (*Zea mays* L.). *Int J Syst Evol Microbiol* 65:4734–4742
- Lee JH, Lee SS (2012) *Leucobacter margaritifformis* sp. nov., isolated from bamboo extract. *Curr Microbiol* 64:441–448
- Lee EP, Han YS, Lee SI, Cho KT, Park JH, You Y (2017) Effect of nutrient and moisture on the growth and reproduction of *Epilobium hirsutum* L., an endangered plant. *J Environ Prot Ecol*. <https://doi.org/10.1186/s41610-017-0054-z>
- Li W, Xu P, Schumann P, Zhang Y, Pukall R, Xu L, Stackebrandt E, Jiang C (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. *Int J Syst Evol Microbiol* 57:1424–1428
- Li Y, Fang W, Xie SJ, Yang X, Wang LF (2016) *Leucobacter corticis* sp. nov., isolated from symptomatic bark of *Populus × euramericana* canker. *Int J Syst Evol Microbiol* 65:432–437
- Li DD, Xu XJ, Yu HW, Han XR (2017) Characterization of Pb²⁺ biosorption by psychrotrophic strain *Pseudomonas* sp. 13 isolated from permafrost soil of Mohe wetland in Northeast China. *J Environ Manage* 196:8–15
- Lin YC, Uemori K, Briel DA, Arunpairojana V, Yokota A (2004) *Zimmermannella helvola* gen. nov., sp. nov., *Zimmermannella alba* sp. nov., *Zimmermannella bifida* sp. nov., *Zimmermannella faecalis* sp. nov. and *Leucobacter albus* sp. nov., novel members of the family *Microbacteriaceae*. *Int J Syst Evol Microbiol* 54:1669–1676
- Lucia P, Valentin N, Cristina H, Denisa IU, Nicorescu I (2015) Antimicrobial and synergistic activity of some whole and selective *Epilobium hirsutum* L. (Great Willowherb) extracts tested on standard and wild *staphylococcus aureus* strains. *Farmacia* 63:690–695
- Martin E, Lidders N, Jackel U, Schumann P, Kampfer P (2010) *Leucobacter aerolatus* sp. nov., from the air of a duck barn. *Int J Syst Evol Microbiol* 60:2838–2842
- Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 39:159–167
- Minnikin DE, Collins MD, Goodfellow M (1979) Fatty acid and IPolar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Microbiol* 47:87–95
- Morais PV, Francisco R, Branco R, Chung AP, Da CM (2004) *Leucobacter chromiireducens* sp. nov. and *Leucobacter aridicollis* sp. nov., two new species isolated from a chromium contaminated environment. *Syst Appl Microbiol* 27:646–652
- Morais PV, Francisco R, Branco R, Chung AP, Da CM (2005) Validation of publication of new names and new

- combinations previously effectively published outside the IJSEM. *Int J Syst Evol Microbiol* 55:547–549
- Morais PV, Paulo C, Francisco R, Branco R, Chung AP, Costa MS (2006) *Leucobacter luti* sp. nov., and *Leucobacter alluvii* sp. nov., two new species of the genus *Leucobacter* isolated under chromium stress. *Syst Appl Microbiol* 29:414–421
- Muir RE, Tan MW (2007) *Leucobacter chromiireducens* subsp. *solipictus* subsp. nov., a pigmented bacterium isolated from the nematode *Caenorhabditis elegans*, and emended description of *L. chromiireducens*. *Int J Syst Evol Microbiol* 57:2770–2776
- Ou XH, Wang L, Guo LP, Cui XM, Liu DH, Yang Y (2016) Soil-plant metal relations in *Panax notoginseng*: an ecosystem health risk assessment. *Int J Environ Res Public Health* 13:1089–1093
- Rosselló-Móra R, Trujillo ME, Sutcliffe IC (2017) Introducing a Digital Protologue: a timely move towards a database-driven systematics of Archaea and Bacteria. *Syst Appl Microbiol* 40:121–122
- Rui YK, Shen JB, Zhang FS (2008) Application of ICP-MS to determination of heavy metal content of heavy metals in two kinds of N fertilizer. *Spectrosc Spectr Anal* 10:2425–2427
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sarangi A, Krishnan C (2008) Comparison of in vitro Cr(VI) reduction by CFEs of chromate resistant bacteria isolated from chromate contaminated soil. *Biores Technol* 99:4130–4137
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI technical note 101. MIDI Inc., Newark
- Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 36:407–477
- Schumann P, Pukall R (2017) *Leucobacter weissii* sp. nov., an isolate from activated sludge once described as first representative of the peptidoglycan variation B2δ, and emended description of the genus *Leucobacter*. *Int J Syst Evol Microbiol* 67:5244–5251
- Shin NR, Kim MS, Jung MJ, Roh SW, Nam YD, Park EJ, Bae JW (2011) *Leucobacter celer* sp. nov., isolated from Korean fermented seafood. *Int J Syst Evol Microbiol* 61:2353–2357
- Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313–340
- Sturm G, Jacobs J, Sproer C, Schumann P, Gescher J (2011) *Leucobacter chromiireducens* sp. nov., a chromate-resistant strain. *Int J Syst Evol Microbiol* 61:956–960
- Sudhir K, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Evol Genet Anal* 33:1870–1874
- Sun LN, Pan DD, Wu XW, Yang ED, Hua RM, Li QX (2017) *Leucobacter triazinivorans* sp. nov., a s-triazine herbicide prometryn-degrading bacterium isolated from sludge. *Int J Syst Evol Microbiol* 68:204–210
- Takeuchi M, Weiss N, Schumann P, Yokota A (1996) *Leucobacter komagatae* gen. nov., sp. nov., a new aerobic gram-positive, nonsporulating rod with 2,4-diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* 46:967–971
- Tang S, Wang Y, Chen Y, Lou K, Cao L, Xu L, Li W (2009) *Zhihengliuella alba* sp. nov., and emended description of the genus *Zhihengliuella*. *Int J Syst Evol Microbiol* 59:2025–2031
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882
- Toth BH, Blazics B, Kéry A (2008) Polyphenol composition and antioxidant capacity of *Epilobium* species. *J Pharm Biomed Anal* 49:26–31
- Weon HY, Anandham R, Tamura T, Hamada M, Kim SJ, Kim YS, Suzuki KI, Kwon SW (2012) *Leucobacter denitrificans* sp. nov., isolated from cow dung. *J Microbiol* 50:161–165
- Williams RT, Crawford RL (1983) Microbial diversity of Minnesota peatlands. *Microb Ecol* 9:201–214
- Xu P, Li WJ, Tang SK, Zhang YQ, Chen GZ, Chen HH, Xu LH, Jiang CL (2005) *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family ‘*Oxalobacteraceae*’ isolated from China. *Int J Syst Evol Microbiol* 55:1149–1153
- Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617
- Yun J, Roh SW, Kim M, Jung M, Park E, Shin K, Nam Y, Bae J (2011) *Leucobacter salsicius* sp. nov., from a salt-fermented food. *Int J Syst Evol Microbiol* 61:502–506
- Zhu D, Zhang PP, Li PP, Wu J, Xie CX, Sun JZ, Niu LL (2016) Description of *Leucobacter holotrichiae* sp. nov., isolated from the gut of *Holotrichia obliata* larvae. *Int J Syst Evol Microbiol* 66:1857–1861