Leucobacter chromiireducens subsp. *solipictus* subsp. nov., a pigmented bacterium isolated from the nematode *Caenorhabditis elegans*, and emended description of *L. chromiireducens*

Rachel E. Muir¹ and Man-Wah Tan^{1,2}

Correspondence

Man-Wah Tan

mwtan@stanford.edu

¹Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305-5120, USA

²Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5120, USA

A yellow-pigmented, Gram-positive, aerobic, non-motile, non-spore-forming, irregular rod-shaped bacterium (strain TAN 31504^T) was isolated from the bacteriophagous nematode *Caenorhabditis elegans*. Based on 16S rRNA gene sequence similarity, DNA G+C content of 69.5 mol%, 2,4-diaminobutyric acid in the cell-wall peptidoglycan, major menaquinone MK-11, abundance of anteiso- and iso-fatty acids, polar lipids diphosphatidylglycerol and phosphatidylglycerol and a number of shared biochemical characteristics, strain TAN 31504^T was placed in the genus *Leucobacter*. DNA–DNA hybridization comparisons demonstrated a 91 % DNA–DNA relatedness between strain TAN 31504^T and *Leucobacter chromiireducens* LMG 22506^T indicating that these two strains belong to the same species, when the recommended threshold value of 70 % DNA–DNA relatedness for the definition of a bacterial species by the ad hoc committee on reconciliation of approaches to bacterial systematics is considered. Based on distinct differences in morphology, physiology, chemotaxonomic markers and various biochemical characteristics, it is proposed to split the species *L. chromiireducens* into two novel subspecies, *Leucobacter chromiireducens* subsp. *chromiireducens* subsp. nov. (type strain L-1^T=CIP 108389^T=LMG 22506^T) and *Leucobacter chromiireducens* subsp. *solipictus* subsp. nov. (type strain TAN 31504^T=DSM 18340^T=ATCC BAA-1336^T).

The original description of *Leucobacter chromiireducens* (Morais *et al.*, 2004) as a novel species of the genus *Leucobacter* and the validation of the species name (Morais *et al.*, 2005) have been published previously. This work describes a strain isolated from the nematode *Caenorhabditis elegans*, which appears to be the first pigmented member of the genus *Leucobacter*, and presents a detailed comparison of the newly isolated organism with its closest phylogenetic relative.

Strain TAN 31504^T was isolated from infected *C. elegans* that unexpectedly were found to have accumulated yellow-pigmented bacteria within their translucent bodies when feeding on cream-coloured lawns of *Salmonella enterica*

serovar Typhimurium SL1344. Three infected nematodes were disrupted within a small aliquot of Luria–Bertani (LB) medium and subsequently plated for single colonies on LB agar. Following 2 days incubation at 25 °C, colonies of the contaminant(s) were easily discernible from SL1344 by their yellow pigmentation. A single yellow colony was restreaked onto solid media for purity verification, and was confirmed to be axenic. The LB medium comprised: 10 g tryptone, 5 g yeast extract, 10 g NaCl, in 1 l H₂O; pH 7. The type strain of *L. chromiireducens* (LMG 22506^T) was obtained from the BCCM/LMG, Laboratorium voor Microbiologie, Universiteit Gent, Belgium.

Experiments were performed at least twice; standard errors of the mean are reported only for results obtained from three or more replicates. Cells were grown in the presence of approximately 25 µmol m⁻² s⁻¹ white light in either liquid or on solid (1.5 % agar) LB medium under aerobic conditions at 25 °C, unless otherwise indicated. Optical densities were measured at 600 nm. Growth rate studies were performed with 30 ml liquid cultures inoculated with 30 µl saturated (optical density at 600 nm=~3.0) cultures grown overnight at 25 °C in 250 ml Pyrex Erlenmeyer

Abbreviation: DAB, 2,4-diaminobutyric acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TAN 31504^T is DQ845457.

Transmission electron micrographs of cells of strain TAN 31504^T and a neighbour-joining tree based on 16S rRNA gene sequences showing the relationship of strain TAN 31504^T to members of the genus *Leucobacter* and other reference organisms are available as supplementary figures with the online version of this paper.

flasks shaken at approximately 250 r.p.m. The pH range for growth was determined in liquid medium that was adjusted to pH 5 using glacial acetic acid, to pH 5.5 and 6 with MES, pH 6.5 with MOPS, pH 7.5 and 8 with HEPES, and pH 8.5 and 9 with Tris base. Halotolerance was examined in liquid cultures containing NaCl at concentrations of 0–10 % (w/v; 1 % increments). For growth in the presence of toxic metals or antibiotics (excluding fosmidomycin and mevinolin), solid medium was supplemented to the final concentrations indicated in Table 1. The effects of the isoprenoid synthesis inhibitors fosmidomycin and mevinolin were assessed in liquid culture (Trutko *et al.*, 2005). The ability to reduce hexavalent chromium and to exhibit β -galactosidase activity was determined as described by Greenberg *et al.* (1998) and Miller (1972). A GP2 MicroPlate (Biolog) was used, according to the manufacturer's instructions, to characterize the utilization of the 95 carbon sources specified in Table 1. The GP2 MicroPlate reactions were scored visually after 24 h incubation at 25 °C. All wells that developed a noticeable purple colour were scored as positive for utilization of the substrate harboured. Wells that remained colourless were scored as negative. The well reactions were characterized as follows: dark purple, strong; light purple, moderate; faint purple, weak; colourless, negative (Table 1). The ability to ferment carbohydrate was determined after 7 days incubation at 25 °C in liquid LB medium plus the pH indicator phenol red. Collection of gas within a Durham tube and/or the generation of an acidic pH within the media was taken to indicate carbohydrate fermentation. The absence of bubbles in the Durham tube and an unchanged or increased pH indicated a negative test result for fermentation. The test for anaerobic growth in LB medium was performed in 10 ml anaerobic jars with rubber septa (Sigma) that were evacuated with hydrogen gas and then incubated at 25 °C for 7 days. Standard methods were used to assess all other biochemical characteristics (Gerhardt *et al.*, 1981). Cell morphology and motility were determined by using phase-contrast microscopy and negative staining transmission electron microscopy (see Supplementary Fig. S1, available in IJSEM Online). Swimming, swarming and twitching motility was further assessed with motility agar. Pigment accumulation was examined under varied light quality using LEDs for specific wavelengths (www.superbrightleds.com). Pigment was extracted with 100–200 μ l methanol at 65 °C from wet cell pellets generated from 1.5 ml saturated overnight cultures. A Plasmid Mini kit (Qiagen) was used for the detection of endogenous plasmids.

The peptidoglycan and cell-wall sugars were isolated and the types and corresponding structures determined (MacKenzie, 1987; Schleifer & Kandler, 1972; Schleifer, 1985; Rhuland *et al.*, 1955; Stanek & Roberts, 1974). Briefly, purification of the cell wall and preparation of the cell-wall hydrolysates were done according to the methods of Schleifer & Kandler (1972). The N-terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described

by Schleifer (1985). For the cellular fatty acid profile, fatty acid methyl esters were prepared, separated and identified using the MIDI Sherlock Microbial Identification System (Microbial ID, Inc.) as described by Vandamme *et al.* (1992). Analyses of respiratory quinones and polar lipids were carried out as described by Tindall (1989, 1990).

Isolation and purification of chromosomal DNA were performed as described by Cashion *et al.*, (1977) and Visuvanathan *et al.* (1989). The protocol used for the determination of the DNA G+C content by HPLC was adapted from Tamaoka & Komagata (1984) and the deoxyribonucleotides analysed by HPLC were generated according to the method of Mesbah & Whitman (1989). The percentage DNA–DNA relatedness was determined using the methods described by De Ley *et al.* (1970), with the modifications detailed by Huß *et al.* (1983).

Genomic DNA was isolated and the 16S rRNA gene amplified essentially as described by Ausubel *et al.* (1989). The PCR product was sequenced and analysed by Sequetech Corporation. CLUSTAL W (Thompson *et al.*, 1994) was used for the alignment of sequences and the generation of pairwise alignment scores (Wilbur & Lipman, 1983). SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package (Felsenstein, 2006) were used to produce the phylogenetic consensus tree (see Supplementary Fig. S2, in IJSEM Online).

Strain TAN 31504^T grew aerobically, but not under anaerobic conditions. Cells did not ferment any of the carbon sources present in LB medium, but could produce basic ions from the metabolism of LB medium substrates, generating a pH of >9.6 in spent media. The utilizable substrates and the pH and growth temperature ranges of strain TAN 31504^T are given in Table 1. The optimum growth rate of exponentially growing cultures occurred between 25 and 32 °C. No growth was observed after 30 days at 4 °C, but was readily apparent after 7 days at 10 °C. A notable decrease in growth occurred at temperatures above 34 °C, with marginal growth occurring at 37 °C and no growth at 40 °C. Cultivation temperature affected pigment accumulation and the number of colony forming units persisting in stationary phase cultures. More colonies were generated from cultures grown to saturation at 25–28 °C than at either 15 or 34 °C. Collectively, the results indicated that strain TAN 31504^T grew best at 25 °C; this growth temperature was used in subsequent experiments.

Detailed characteristics of strain TAN 31504^T are given in the species description. Briefly, cells grown in liquid culture tended to be shorter rods than cells grown on solid medium (see Supplementary Fig. S1, in IJSEM Online). In liquid culture, the cell span was restricted to no more than two cell lengths. On solid media, cells formed chains of short and long rods that varied from two up to, and less frequently exceeding, six cell lengths. Cells were non-motile. Neither flagella nor pili were detected in electron micrographs taken at >112 000 times magnification of

Table 1. Diagnostic and differential characteristics that support a subspecies relationship between strain TAN 31504^T and *L. chromiireducens* LMG 22506^T

Both strains are negative for the utilization of α -cyclodextrin, β -cyclodextrin, dextrin, glycogen, inulin, mannan, *N*-acetyl-D-glucosamine, *N*-acetyl- β -D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, D-cellobiose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α -D-glucose, *myo*-inositol, α -D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, methyl α -D-galactoside, 3-methyl D-glucose, methyl α -D-glucoside, methyl β -D-glucoside, methyl α -D-mannoside, palatinose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketoglutaric acid, α -ketovaleric acid, lactamide, D-lactic acid, L-lactic acid, D-malic acid, L-malic acid, succinic acid monomethyl ester, propionic acid, pyruvic acid, succinic acid, *N*-acetyl-L-glutamic acid, D-alanine, L-asparagine, L-pyroglutamic acid, 2,3-butanediol, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, uridine 5'-monophosphate, D-fructose 6-phosphate, α -D-glucose 1-phosphate, D-glucose 6-phosphate and DL- α -glycerol phosphate. Both strains had identical profiles for all other biochemical tests described in the text that are not listed here.

Characteristic	Strain TAN 31504 ^T	<i>L. chromiireducens</i> LMG 22506 ^T
Chemotaxonomic		
DNA G + C content (mol%)	69.5	66.7*
Menaquinone MK-11 (% of total)	62	80*
Menaquinone MK-10 (% of total)	38	20*
Cellular fatty acids (% of total)		
anteiso-C _{15:0}	66	54*
anteiso-C _{17:0}	14	16*
iso-C _{16:0}	11	12*
linear C _{16:0}	4	12*
Polar lipids†	DPG, PG, unknown glycolipid	NA
Peptidoglycan cell-wall amino acid ratio		
DAB:Ala:Thr:Gly:Glu (%)‡	0.5:1.8:0.6:1:1	1:2.1:0.7:1.4:1.1*
Metabolic substrates§		
Tween 80	Moderate	Strong
Acetic acid	None	Weak
Succinamic acid	Weak	None
L-Alanyl glycine	Moderate	Strong
Glycyl L-glutamic acid	Moderate	Strong
Glycerol	Strong	None
Adenosine	None	Strong
2'-Deoxyadenosine	None	Moderate
Inosine	None	Weak
Thymidine	None	Moderate
Growth in the presence of: 		
50–300 µg streptomycin ml ⁻¹ ¶	Resistant	Sensitive
Toxic metals¶ #		
5 mM hexavalent chromium	+	++
10 mM hexavalent chromium	+/-	++
1 mM arsenite	+	++
10 mM arsenite	-	++
9% NaCl (w/v)**	No growth	Poor growth
pH range**	5.5–9	5–9
pH optimum**	7.5–8.5	7*
Temperature range (°C)††	10–37	4–37
Temperature optimum (°C)**	25–32	28*
Other phenotypic characteristics 		
Pigmentation at ≤25 °C	Yellow, light-induced	None
Bacterial lawn quality	Extremely hydrophobic	Moderately hydrophobic
Growth rate (doubling time) (min)	102.3 ± 6.8	80.3 ± 6.5

*Data from Morais *et al.* (2004).

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; NA, not available.

‡The B2δ-type variant peptidoglycan structure of both strains is characterized by the cell-wall diamino acid L-DAB.

§GP2 MicroPlate colorimetric assay for utilization of substrate; reactions scored as strong, moderate, weak or none (negative). Both strains were

Table 1. cont.

scored as moderate on pyruvic acid methyl ester and were scored as strong on Tween 40, *p*-hydroxyphenylacetic acid, L-alaninamide, L-alanine, L-glutamic acid, L-serine and putrescine.

||Growth conditions and experimental procedures are indicated in the text.

¶Growth was assessed after 5 days incubation at 25 °C.

#Growth scored as ++, good; +, moderate; +/-, poor; -, none. Both strains were scored as good on 1 mM hexavalent chromium and 80 µM cadmium; only strain LMG 22506^T exhibited the ability to reduce hexavalent chromium.

**Growth rates were assessed during exponential growth phase (optical density at 600 nm=0.2–1.0). Both strains grew well in 3 % NaCl and exhibited moderate growth in 6 % NaCl.

††Temperature range for growth was assessed on solid LB medium.

liquid or solid grown cells; however, in electron micrographs of both culture types an extracellular material joining cells to one another was observed (Supplementary Fig. S1 in IJSEM Online). Lawns of cells generated on LB agar were extremely hydrophobic and formed a hardened gel-like film with extended incubation periods.

Strain TAN 31504^T expressed a yellow light-inducible pigment that was excluded from crude cytosolic fractions and was methanol soluble. The absorption spectrum of the methanol-solubilized pigment suggested that it was the C₄₀-carotenoid neurosporene (Lee & Schmidt-Dannert, 2002; Trutko *et al.*, 2005). Carotenogenesis was dependent on light quality and temperature. Pigmentation occurred in full spectrum white light, 5–10 µmol m⁻² s⁻¹ blue light and 6–10 µmol m⁻² s⁻¹ yellow light, but not in 10 µmol m⁻² s⁻¹ red light. Cells grown in white light at permissive temperatures above 25 °C failed to accumulate the yellow pigment; at 10–15 °C cells accumulated more pigment and were much brighter yellow than at 20–25 °C. The isoprenoid synthesis inhibitors fosmidomycin (nonmevalonate pathway inhibitor) and mevinolin (mevalonate pathway inhibitor) affected both the growth rate and pigment accumulation of strain TAN 31504^T. Fosmidomycin at 2.0 mM caused a significant decrease in growth rate, but allowed cells to retain pigment. Mevinolin at 1.0 mM also failed to cause a complete loss of pigmentation and did not reduce the quantities of cell mass accumulated to amounts below those achieved in 0 mM mevinolin cultures. Significant reductions in both growth rate and pigment accumulation were achieved in LB containing a combination of 0.5 mM fosmidomycin and 0.5 mM mevinolin, suggesting that strain TAN 31504^T possessed both types of isoprenoid biosynthetic pathway (Takagi *et al.*, 2000; Trutko *et al.*, 2005).

The major isoprenoid quinones of strain TAN 31504^T were MK-11 (62 %) and MK-10 (38 %). The most abundant cellular fatty acids were anteiso-C_{15:0} (66 %), anteiso-C_{17:0} (14 %), iso-C_{16:0} (11 %), and linear C_{16:0} (4 %). Galactose was the only cell-wall sugar detected. The polar lipids present in the strain included diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid, which was suggested to be a diglycosyl diglyceride based on the *R_f* value. The DNA G+C content of strain TAN 31504^T was 69.5 mol%.

The amino acids present in the cell wall of strain TAN 31504^T were 2,4-diaminobutyric acid (DAB), alanine, threonine, glycine and glutamic acid in an approximate molar ratio of 0.5:1.8:0.6:1.0:1.0. These ratios were similar to those of other *Leucobacter* species (Morais *et al.*, 2004, 2006). The N-terminus of the interpeptide bridge of strain TAN 31504^T was occupied by an alanine, not the threonine residue common to the previously reported B2δ-type structure (Hensel, 1984). The peptidoglycan structure was therefore determined to be a rare B2δ-type variant that was common to *L. chromiireducens*, *Leucobacter luti* and *Leucobacter alluvii* (Morais *et al.*, 2004, 2006). The chemotaxonomic parameters thus far listed were all in accordance with the placement of strain TAN 31504^T within the genus *Leucobacter* (Takeuchi *et al.*, 1996; Lin *et al.*, 2004; Morais *et al.*, 2004, 2006).

A nearly complete 16S rRNA gene sequence, encompassing 1508 bases, was obtained for strain TAN 31504^T. The sequence was aligned with those of other members of the genus *Leucobacter* (Takeuchi *et al.*, 1996; Lin *et al.*, 2004; Morais *et al.*, 2004, 2006) and pairwise alignment scores were generated. From the comparison, the 16S rRNA gene of strain TAN 31504^T was most similar to that of *L. chromiireducens*, sharing approximately 99.5 % sequence similarity. Using an implemented neighbour-joining method (Felsenstein, 2006; Saitou & Nei, 1987), a phylogenetic tree was constructed from the 16S rRNA gene sequences of strain TAN 31504^T and those of other *Leucobacter* species and various members of the family *Microbacteriaceae* that had been shown previously to cluster along a common phylogenetic branch (Kämpfer *et al.*, 2000; Männistö *et al.*, 2000; Behrendt *et al.*, 2002). The results indicated, with a 100 % bootstrap confidence level, that strain TAN 31504^T belonged to the genus *Leucobacter*, displaying the least evolutionary distance from *L. chromiireducens* (see Supplementary Fig. S2 in IJSEM Online).

The level of DNA–DNA relatedness between strain TAN 31504^T and *L. chromiireducens* LMG 22506^T was assessed in duplicate DNA–DNA hybridization assays. The mean DNA–DNA relatedness for the two strains was calculated to be approximately 91 %. When the threshold value of 70 % DNA–DNA relatedness for the definition of a bacterial species recommended by the ad hoc committee

on reconciliation of approaches to bacterial systematics is considered (Wayne *et al.*, 1987), strain TAN 31504^T could be regarded as representing the species *L. chromiireducens*. To further distinguish strain TAN 31504^T, extensive morphological, physiological and biochemical comparisons of *L. chromiireducens* strain LMG 22506^T and strain TAN 31504^T were performed. The data obtained from the comparative studies are summarized in Table 1.

Strain TAN 31504^T and *L. chromiireducens* LMG 22506^T exhibited similar metabolic profiles (Table 1). In minor disagreement with that reported by Morais *et al.* (2004), strain LMG 22506^T did not utilize L-rhamnose or glycerol, whereas strain TAN 31504^T metabolized glycerol readily. Both strains did not produce urease or hydrolyse starch. The previous report of urease production by strain LMG 22506^T (Morais *et al.*, 2004) could be attributed to the alkalization of the urease test growth media in the absence of urea that both strains caused. A number of other carbon sources that were reported to be weakly assimilated by strain LMG 22506^T (Morais *et al.*, 2004) were not confirmed by the use of the Biolog GP2 MicroPlate assay in three replicate experiments.

Strains TAN 31504^T and LMG 22506^T shared the same optimum growth temperature, a similar pH range and a comparable tolerance to salt (Table 1; Morais *et al.*, 2004). Both strains lacked endogenous plasmids and were resistant to various levels of chromium, cadmium and arsenite, and a number of antibiotics (see species descriptions and Table 1). Unlike strain LMG 22506^T, strain TAN 31504^T could not reduce hexavalent chromium to the less toxic trivalent oxidation state. Cells of strain LMG 22506^T remained cream-coloured under all conditions tested, produced moderately hydrophobic lawns and exhibited a faster doubling rate than strain TAN 31504^T.

Phylogenetic and chemotaxonomic characterization indicated that strain TAN 31504^T was distinct from *L. chromiireducens* and that the two strains represent separate subspecies. Therefore, strain TAN 31504^T is proposed to represent a novel subspecies with the name *Leucobacter chromiireducens* subsp. *solipictus* subsp. nov., with the concomitant creation of *Leucobacter chromiireducens* subsp. *chromiireducens* subsp. nov.

Emended description of *Leucobacter chromiireducens* Morais *et al.* 2005

The characteristics are essentially the same as those described for the genus (Takeuchi *et al.*, 1996), differing in the G+C content of the DNA and in the abundance of menaquinones. Additional biochemical characteristics of the species to those reported by Morais *et al.* (2004) are given in Table 1. Contains two chromium-tolerant subspecies, one that is non-pigmented and can reduce hexavalent chromium to trivalent chromium and another that is pigmented and cannot reduce hexavalent chromium.

Description of *Leucobacter chromiireducens* subsp. *solipictus* subsp. nov.

Leucobacter chromiireducens subsp. *solipictus* (so.li.pic'tus. L. masc. n. *sol* the sun; L. masc. part. adj. *pictus* painted; L. masc. part. adj. *solipictus* painted by the sun).

Cells are Gram-positive, aerobic, non-motile, non-spore-forming irregular rods that are 0.4–0.6 µm wide and 0.8–1.4 µm long when grown at 25 °C in liquid LB medium and 0.5–0.7 µm wide and 0.8–4.3 µm long when grown at 25 °C on solid LB agar for 1–2 days. Cells produce an extracellular material. Colonies are circular, entire, convex, small (0.5–1 mm in diameter), smooth, glistening, yellow-pigmented when grown in the light or cream-coloured when grown in the dark, and opaque on LB agar after 2–5 days growth at 25 °C. The light-induced pigment is soluble in methanol and displays characteristic peaks in absorption at 413, 436 and 466 nm. Pigment production is dependent on light quality and temperature. Depigmentation of cells occurs in the combined presence of 0.5 mM fosmidomycin and 0.5 mM mevinolin. Growth occurs at 10–37 °C, pH 5.5–9, in LB containing 0–8% NaCl. Growth does not occur at 4 or 40 °C, pH 5 or in the presence of ≥9% NaCl. Optimum growth occurs between 25 and 32 °C at pH 7.5–8.5 in aerated LB lacking salt. After 2–5 days incubation at 25 °C, growth can be seen on solid LB agar containing each of the following individually: 1 mM arsenite, 80 µM cadmium and 5 mM hexavalent chromium; however, cells are unable to reduce hexavalent chromium to trivalent chromium. Cells are resistant to 50 µg kanamycin ml⁻¹, 12.5 µg tetracycline ml⁻¹, 10 µg gentamicin ml⁻¹, 300 µg streptomycin ml⁻¹ and 20 µg chloramphenicol ml⁻¹ and sensitive to 100 µg ampicillin ml⁻¹ and 100 µg rifampicin ml⁻¹. Positive for catalase and alkaline phosphatase activities, but not for cytochrome *c* oxidase, nitrate reductase, β-galactosidase, urease, gelatinase, amylase, cysteine desulfurase, tryptophanase, phenylalanine deaminase, haemolytic or lipase activities. Carbon source utilization profile is given in Table 1. No endogenous plasmids are present. Cell-wall peptidoglycan contains DAB, alanine, glutamic acid, glycine and threonine. The peptidoglycan structure is a B2δ-type variant. Galactose is the only cell-wall sugar detected. Major isoprenoid quinones are MK-11 and MK-10. Major cellular fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and linear C_{16:0}. Polar lipids include diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid. The G+C content of the DNA of the type strain is 69.5 mol%.

The type strain is TAN 31504^T (=DSM 18340^T=ATCC BAA-1336^T), which was isolated from *Caenorhabditis elegans* and appeared as a contaminant. The location of isolation is designated Stanford, CA, USA.

Description of *Leucobacter chromiireducens* subsp. *chromiireducens* Morais *et al.* 2005, subsp. nov.

The description is essentially as given by Morais *et al.* (2004). In addition, growth occurs at 4 °C. After 2–5 days

incubation at 25 °C, growth can be seen on solid LB agar containing each of the following individually: 1 mM arsenite, 80 µM cadmium, 50 µg kanamycin ml⁻¹, 12.5 µg tetracycline ml⁻¹, 10 µg gentamicin ml⁻¹ and 20 µg chloramphenicol ml⁻¹; cells are sensitive to 50 µg streptomycin ml⁻¹, 100 µg ampicillin ml⁻¹ and 100 µg rifampicin ml⁻¹. Negative for β-galactosidase, urease, amylase, cysteine desulfurase, tryptophanase, phenylalanine deaminase, haemolytic and lipase activities. Additional characteristics of the carbon source utilization profile are given in Table 1. No endogenous plasmids are present.

The type strain is L-1^T (=CIP 108389^T=LMG 22506^T).

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