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

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A yellow-pigmented, Gram-positive, aerobic, non-motile, non-spore-forming, irregular rod-shaped bacterium (strain TAN 31504ᵀ) was isolated from the bacteriophage 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 31504ᵀ was placed in the genus Leucobacter. DNA–DNA hybridization comparisons demonstrated a 91 % DNA–DNA relatedness between strain TAN 31504ᵀ and Leucobacter chromiireducens LMG 22506ᵀ 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 Leucobacter chromiireducens into two novel subspecies, Leucobacter chromiireducens subsp. chromiireducens subsp. nov. (type strain L-1ᵀ=CIP 108389ᵀ=LMG 22506ᵀ) and Leucobacter chromiireducens subsp. solipictus subsp. nov. (type strain TAN 31504ᵀ=DSM 18340ᵀ=ATCC BAA-1336ᵀ).

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 31504ᵀ 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 22506ᵀ) 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 flasks.
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 µl 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; Staneck & 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 Viswanathan 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 31504T 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 31504T 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 31504T grew best at 25 °C; this growth temperature was used in subsequent experiments.

Detailed characteristics of strain TAN 31504T 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 31504T and L. chromiireducens LMG 22506T

Both strains are negative for the utilization of α-cyclodextrin, β-cyclodextrin, dextrin, glycogen, inulín, mannan, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, amyladín, L-arabinose, D-arabitol, arbutín, D-cellobiose, D-fructose, D-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-glucan, α-D-glucose, α-mannopyranoside, D-mannose, D-melezitose, methyl α-D-galactoside, 3-methyl α-D-glucoside, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, palatinose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulose, D-sorbitol, stachyose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylitol, 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, pyruvíc acid, succinic acid, N-acetyl-D-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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain TAN 31504T</th>
<th>L. chromiireducens LMG 22506T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemotaxonomic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69.5</td>
<td>66.7*</td>
</tr>
<tr>
<td>Menaquinone MK-11 (% of total)</td>
<td>62</td>
<td>80*</td>
</tr>
<tr>
<td>Menaquinone MK-10 (% of total)</td>
<td>38</td>
<td>20*</td>
</tr>
<tr>
<td>Cellular fatty acids (% of total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>anteiso-C₁₅ : 0</td>
<td>66</td>
<td>54*</td>
</tr>
<tr>
<td>anteiso-C₁₇ : 0</td>
<td>14</td>
<td>16*</td>
</tr>
<tr>
<td>iso-C₁₆ : 0</td>
<td>11</td>
<td>12*</td>
</tr>
<tr>
<td>linear C₁₆ : 0</td>
<td>4</td>
<td>12*</td>
</tr>
<tr>
<td><strong>Polar lipids†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DPG, PG, unknown glycolipid</td>
<td></td>
</tr>
<tr>
<td>Peptidoglycan cell-wall amino acid ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAB : Ala : Thr : Gly : Glu (%)</td>
<td>0.5 : 1.8 : 0.6 : 1 : 1</td>
<td>1 : 2.1 : 0.7 : 1.4 : 1.1*</td>
</tr>
<tr>
<td><strong>Metabolic substrates§</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>Moderate</td>
<td>Strong</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>None</td>
<td>Weak</td>
</tr>
<tr>
<td>Succinamic acid</td>
<td>Weak</td>
<td>None</td>
</tr>
<tr>
<td>L-Alanine glycine</td>
<td>Moderate</td>
<td>Strong</td>
</tr>
<tr>
<td>Glycyl L-glutamic acid</td>
<td>Moderate</td>
<td>Strong</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Strong</td>
<td>None</td>
</tr>
<tr>
<td>Adenosine</td>
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<td>Strong</td>
</tr>
<tr>
<td>2′-Deoxyadenosine</td>
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<td>Moderate</td>
</tr>
<tr>
<td>Inosine</td>
<td>None</td>
<td>Weak</td>
</tr>
<tr>
<td>Thymidine</td>
<td>None</td>
<td>Moderate</td>
</tr>
<tr>
<td>**Growth in the presence of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–300 μg streptomycin ml⁻¹ ¶</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Toxic metals§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM hexavalent chromium</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 mM hexavalent chromium</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td>1 mM arsenite</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 mM arsenite</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>9 % NaCl (w/v)**</td>
<td>No growth</td>
<td>Poor growth</td>
</tr>
<tr>
<td>pH range**</td>
<td>5.5–9</td>
<td>5–9</td>
</tr>
<tr>
<td>pH optimum**</td>
<td>7.5–8.5</td>
<td>7*</td>
</tr>
<tr>
<td>Temperature range (°C)††</td>
<td>10–37</td>
<td>4–37</td>
</tr>
<tr>
<td>Temperature optimum (°C)**‡‡</td>
<td>25–32</td>
<td>28*</td>
</tr>
<tr>
<td>**Other phenotypic characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigmentation at ≤25 °C</td>
<td>Yellow, light-induced</td>
<td>None</td>
</tr>
<tr>
<td>Bacterial lawn quality</td>
<td>Extremely hydrophobic</td>
<td>Moderately hydrophobic</td>
</tr>
<tr>
<td>Growth rate (doubling time) (min)</td>
<td>102.3 ± 6.8</td>
<td>80.3 ± 6.5</td>
</tr>
</tbody>
</table>

*Data from Morais et al. (2004).
†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; NA, not available.
‡The B₂₈ type variant peptidoglycan structure of both strains is characterized by the cell-wall diaminoc acid l-DAB.
§GP2 MicroPlate colorimetric assay for utilization of substrate; reactions scored as strong, moderate, weak or none (negative). Both strains were
Growth conditions and experimental procedures are indicated in the text.

Growing at temperatures above 25°C. The DNA G+C content of strain TAN 31504T was 69.5 mol%.

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 22506T 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 31504T 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, 2004, 2006). The peptidoglycan structure was therefore determined to be a rare B2δ-type variant that was common to L. chro-miireducens, 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 31504T 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 31504T. 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 31504T was most similar to that of L. chro-miireducens, 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 31504T 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; Mannisto et al., 2000; Behrendt et al., 2002). The results indicated, with a 100% bootstrap confidence level, that strain TAN 31504T belonged to the genus Leucobacter, displaying the least evolutionary distance from L. chro-miireducens (see Supplementary Fig. S2 in IJSEM Online).

The level of DNA–DNA relatedness between strain TAN 31504T and L. chro-miireducens LMG 22506T 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 was used for the definition of a bacterial species recommended by the ad hoc committee...

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**Table 1. cont.**

<table>
<thead>
<tr>
<th>Scored as</th>
<th>Pyruvic Acid Methyl Ester</th>
<th>Tween 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

The amino acids present in the cell wall of strain TAN 31504T 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 interpentide bridge of strain TAN 31504T was occupied by an alanine, not the threonine residue common to the previously reported B2δ-type structure (Hensel, 1984). The peptidoglycan structure of strain TAN 31504T was constructed from the 16S rRNA gene sequence of strain TAN 31504T. The 16S rRNA gene sequence was aligned with those of other members of the family Microbacteriaceae that had been shown previously to cluster along a common phylogenetic branch (Kämpfer et al., 2000; Mannisto et al., 2000; Behrendt et al., 2002). The results indicated, with a 100% bootstrap confidence level, that strain TAN 31504T belonged to the genus Leucobacter, displaying the least evolutionary distance from L. chro-miireducens (see Supplementary Fig. S2 in IJSEM Online).

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The major isoprenoid quinones of strain TAN 31504T were MK-11 (62%) and MK-10 (38%). The most abundant cellular fatty acids were anteiso-C15:0 (66%), anteiso-C17:0 (14%), C16:1ω9c (11%) and C16:0 (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 31504T was 69.5 mol%. The amino acids present in the cell wall of strain TAN 31504T 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 interpentide bridge of strain TAN 31504T was occupied by an alanine, not the threonine residue common to the previously reported B2δ-type structure (Hensel, 1984). The peptidoglycan structure of strain TAN 31504T was constructed from the 16S rRNA gene sequence of strain TAN 31504T. The 16S rRNA gene sequence was aligned with those of other members of the family Microbacteriaceae that had been shown previously to cluster along a common phylogenetic branch (Kämpfer et al., 2000; Mannisto et al., 2000; Behrendt et al., 2002). The results indicated, with a 100% bootstrap confidence level, that strain TAN 31504T belonged to the genus Leucobacter, displaying the least evolutionary distance from L. chro-miireducens (see Supplementary Fig. S2 in IJSEM Online).

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

Strain TAN 31504<sup>T</sup> and <i>L. chromiiireducens</i> LMG 22506<sup>T</sup> exhibited similar metabolic profiles (Table 1). In minor disagreement with that reported by Morais et al. (2004), strain LMG 22506<sup>T</sup> did not utilize l-rhamnose or glycerol, whereas strain TAN 31504<sup>T</sup> metabolized glycerol readily. Both strains did not produce urease or hydrolyse starch. The previous report of urease production by strain LMG 22506<sup>T</sup> (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<sup>T</sup> (Morais et al., 2004) were not confirmed by the use of the Biolog GP2 MicroPlate assay in three replicate experiments.

Strains TAN 31504<sup>T</sup> and LMG 22506<sup>T</sup> 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<sup>T</sup>, strain TAN 31504<sup>T</sup> could not reduce hexavalent chromium to the less toxic trivalent oxidation state. Cells of strain LMG 22506<sup>T</sup> remained cream-coloured under all conditions tested, produced moderately hydrophobic lawns and exhibited a faster doubling rate than strain TAN 31504<sup>T</sup>.

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

**Emended description of Leucobacter chromiiireducens 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 chromiiireducens subsp. solipictus subsp. nov.**

<i>Leucobacter chromiiireducens</i> subsp. <i>solipictus</i> (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-spor-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<sup>–1</sup>, 12.5 μg tetracycline ml<sup>–1</sup>, 10 μg gentamicin ml<sup>–1</sup>, 300 μg streptomycin ml<sup>–1</sup> and 20 μg chloramphenicol ml<sup>–1</sup> and sensitive to 100 μg ampicillin ml<sup>–1</sup> and 100 μg rifampicin ml<sup>–1</sup>. 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<sub>15:0</sub>, anteiso-C<sub>17:0</sub> iso-C<sub>16:0</sub> and linear C<sub>16:0</sub>. 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<sup>T</sup> (=DSM 18340<sup>T</sup> = ATCC BAA-1336<sup>T</sup>), which was isolated from <i>Caenohabditis elegans</i> and appeared as a contaminant. The location of isolation is designated Stanford, CA, USA.

**Description of Leucobacter chromiiireducens subsp. chromiiireducens 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-1T (=CIP 108389T=LMG 22506T).

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References


