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 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 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 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 manufac-
turer’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 fermenta-
tion. 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 deter-
mined by using phase-contrast microscopy and negative
staining transmission electron microscopy (see Supple-
mentary 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
methanol at 65 °C from wet cell pellets
generated from cultures grown to saturation.
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 tempera-
tures 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-
nmotile. 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\textsuperscript{T} and \textit{L. chromiireducens} LMG 22506\textsuperscript{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, D-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-glucuronic acid, α-D-glucose, α-xylosidase, lactulose, maltose, maltotriose, D-mannitol, L-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, sedoheptulose, D-stachyose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylene, 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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain TAN 31504\textsuperscript{T}</th>
<th>\textit{L. chromiireducens} LMG 22506\textsuperscript{T}</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-C\textsubscript{15 : 0}</td>
<td>66</td>
<td>54*</td>
</tr>
<tr>
<td>anteiso-C\textsubscript{17 : 0}</td>
<td>14</td>
<td>16*</td>
</tr>
<tr>
<td>iso-C\textsubscript{16 : 0}</td>
<td>11</td>
<td>12*</td>
</tr>
<tr>
<td>linear C\textsubscript{16 : 0}</td>
<td>4</td>
<td>12*</td>
</tr>
<tr>
<td>Polar lipids†</td>
<td>DPG, PG, unknown glycolipid</td>
<td>NA</td>
</tr>
<tr>
<td>Peptidoglycan cell-wall amino acid ratio</td>
<td>DAB: Ala : Thr : Gly : Glu (%)‡</td>
<td>0.5 : 1.8 : 0.6 : 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-Alanyl 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>
<td>None</td>
<td>Strong</td>
</tr>
<tr>
<td>2′-Deoxyadenosine</td>
<td>None</td>
<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 mg streptomycin ml\textsuperscript{−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 \textlessthan_equal 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 \textit{et al.} (2004).†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; NA, not available.§The B2\textsubscript{D}-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 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 C<sub>40</sub>-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<sup>−2</sup> s<sup>−1</sup> blue light and 6–10 μmol m<sup>−2</sup> s<sup>−1</sup> yellow light, but not in 10 μmol m<sup>−2</sup> s<sup>−1</sup> 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 31504T. 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 31504T possessed both types of isoprenoid biosynthetic pathway (Takagi et al., 2000; Trutko et al., 2005).

The major isoprenoid quinones of strain TAN 31504T were MK-11 (62 %) and MK-10 (38 %). The most abundant cellular fatty acids were anteiso-C<sub>15</sub>:0 (66 %), anteiso-C<sub>17</sub>:0 (14 %), iso-C<sub>16</sub>:0 (11 %), and linear C<sub>16</sub>: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<sub>f</sub> 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 interpeptide bridge of strain TAN 31504T was occupied by an alanine, not the threonine residue common to the previously reported B2-type structure (Hensel, 1984). The peptidoglycan structure was therefore determined to be a rare B2-type variant that was common to L. chromiireducens, Leucobacter luti and Leucobacter alluvi (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. 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 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; Männisto 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. chromiireducens (see Supplementary Fig. S2 in IJSEM Online).

The level of DNA–DNA relatedness between strain TAN 31504T and L. chromiireducens 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 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\textsuperscript{T} could be regarded as representing the species \textit{L. chromiiireducens}. To further distinguish strain TAN 31504\textsuperscript{T}, extensive morphological, physiological and biochemical comparisons of \textit{L. chromiiireducens} strain LMG 22506\textsuperscript{T} and strain TAN 31504\textsuperscript{T} were performed. The data obtained from the comparative studies are summarized in Table 1.

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

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

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

**Emended description of \textit{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 \textit{G} + \textit{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 \textit{Leucobacter chromiiireducens} subsp. \textit{solipictus} subsp. nov.**

\textit{Leucobacter chromiiireducens} subsp. \textit{solipictus} (so.li.pic’tus. L. masc. n. sol the sun; L. masc. part. adj. pictus painted; L. masc. part adj. \textit{solipictus} painted by the sun).

Cells are Gram-positive, aerobic, non-motile, non-spore-forming irregular rods that are 0.4–0.6 \textmu m wide and 0.8–1.4 \textmu m long when grown at 25 °C in liquid LB medium and 0.5–0.7 \textmu m wide and 0.8–4.3 \textmu m 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 \textmu M cadmium and 5 mM hexavalent chromium; however, cells are unable to reduce hexavalent chromium to trivalent chromium. Cells are resistant to 50 \textmu g kanamycin ml\textsuperscript{-1}, 12.5 \textmu g tetracycline ml\textsuperscript{-1}, 10 \mu g gentamicin ml\textsuperscript{-1}, 300 \mu g streptomycin ml\textsuperscript{-1} and 20 \mu g chloramphenicol ml\textsuperscript{-1} and sensitive to 100 \mu g ampicillin ml\textsuperscript{-1} and 100 \mu g rifampicin ml\textsuperscript{-1}. Positive for catalase and alkaline phosphatase activities, but not for cytochrome \textit{c} oxidase, nitrate reductase, \textit{β}-galactosidase, urease, gelatinase, amylase, cysteine desulphurase, 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 B2α-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\textsubscript{15:0}, anteiso-C\textsubscript{17:0} isomer C\textsubscript{16:0} and linear C\textsubscript{16:0}. Polar lipids include diphostatidylglycerol, 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\textsuperscript{T} (=DSM 18340\textsuperscript{T}=ATCC BAA-1336\textsuperscript{T}), which was isolated from \textit{Caenorhabditis elegans} and appeared as a contaminant. The location of isolation is designated Stanford, CA, USA.

**Description of \textit{Leucobacter chromiiireducens} subsp. \textit{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
incipient 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


