**Chryseobacterium caeni** sp. nov., isolated from bioreactor sludge

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A Gram-negative, non-spore-forming, yellow-pigmented bacterium, strain N4\textsuperscript{T}, was isolated from a nickel-complexed cyanide-degrading bioreactor and subjected to a polyphasic taxonomic study. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain N4\textsuperscript{T} is affiliated to the genus *Chryseobacterium* of the family *Flavobacteriaceae*. The levels of 16S rRNA gene sequence similarity between strain N4\textsuperscript{T} and the type strains of all known *Chryseobacterium* species were 93.2–95.8\%, suggesting that strain N4\textsuperscript{T} represents a novel species within the genus *Chryseobacterium*. The strain contained iso-C\textsubscript{15}:0 and summed feature 4 as the major fatty acids and menaquinone MK-6 as the predominant respiratory quinone. The G+C content of the genomic DNA was 38.2 mol\%. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain N4\textsuperscript{T} represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium caeni* sp. nov. is proposed. The type strain is N4T (=KCTC 12506\textsuperscript{T} = CCBAU 10201\textsuperscript{T} = DSM 17710\textsuperscript{T}).

The genus *Chryseobacterium* of the family *Flavobacteriaceae* was created by Vandamme *et al.* (1994) to accommodate six species formerly classified within the genus *Flavobacterium*. At present, the genus *Chryseobacterium* comprises 17 species: *Chryseobacterium gleum* (type species), *C. balustinum*, *C. indologenes*, *C. indolotheticum* and *C. scophthalmum* (Vandamme *et al.*, 1994) and the recently described species *C. defluvii* (Kämpfer *et al.*, 2003), *C. joostei* (Hugo *et al.*, 2003), *C. daecheongense* (Kim *et al.*, 2005), *C. formosense* (Young *et al.*, 2005), *C. shigense* (Shimomura *et al.*, 2005), *C. taichungense* (Shen *et al.*, 2005), *C. vrystaatense* (de Beer *et al.*, 2005), *C. hispanicum* (Gallego *et al.*, 2006), *C. piscium* (de Beer *et al.*, 2006), *C. soldanellicola* (Park *et al.*, 2006), *C. taeanense* (Park *et al.*, 2006) and *C. wanjuense* (Weon *et al.*, 2006). ‘*Chryseobacterium proteolyticum*’ was described by Yamaguchi & Yokoe (2000), but this name has not yet been validly published. Two former *Chryseobacterium* species, *C. meningosepticum* (Vandamme *et al.*, 1994) and *C. miricola* (Li *et al.*, 2003), have been transferred to the novel genus *Elizabethkingia* (Kim *et al.*, 2005b).

During a study of bacterial communities associated with the sludge of metal-complexed cyanide treatment bioreactors (Quan *et al.*, 2006), conducted using a culture-dependent approach, a number of bacterial strains were isolated. Strain N4\textsuperscript{T} was isolated from a nickel-complexed cyanide treatment bioreactor. A comparative analysis of 16S rRNA gene sequences indicated that strain N4\textsuperscript{T} was a member of the clade representing the genus *Chryseobacterium*. In order to determine the precise taxonomic position of strain N4\textsuperscript{T}, a polyphasic taxonomic study was carried out.

Strain N4\textsuperscript{T} was cultivated on R2A agar (Difco) at 28 °C for 48 h. Cell biomass for quinone analysis and for DNA extraction was obtained directly from agar plates. For fatty acid methyl ester analysis, strain N4\textsuperscript{T} was cultivated on tryptic soy agar (Difco) at 28 °C for 24 h for direct comparison with reference strains. Cell morphology was examined under a phase-contrast microscope (1000× magnification; Nikon). A Gram reaction was performed as described by Gerhardt *et al.* (1994). Flexirubin-type pigments were detected according to the method of Fautz & Reichenbach (1980). Catalase activity was determined by means of bubble production in a 3\% (v/v) hydrogen peroxide solution. Oxidase activity was determined from the oxidation of 1\% *p*-aminodimethylaniline oxalate. The hydrolysis of starch was tested on starch agar (Difco). Additional enzyme activities, acid production from carbohydrates and the utilization of various substrates as sole carbon sources were determined by using API ZYM, API 20E, API 20NE and API 32GN galleries according to the
DNA was degraded enzymically into nucleotides and (5–42 NaCl concentration (0–5 %), pH (5–10) and temperature (5–42 °C) on growth were determined on R2A agar.

Respiratory quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. For quantitative analysis of the cellular fatty acid composition, a loop of cell mass was harvested and the fatty acids were then saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed by a gas chromatograph (model 6890; Hewlett Packard) and identified by using the Microbial Identification software package (Sasser, 1990). Chromosomal DNA was isolated and purified using a Cell Culture DNA Midi kit (Qiagen) according to the manufacturer’s protocol. For the determination of G+C content, DNA was degraded enzymically into nucleotides and analysed by reversed-phase HPLC as described by Mesbah et al. (1989). Non-methylated λ-phage DNA (Sigma) was used as a calibration reference.

The 16S rRNA gene was amplified by a PCR using two universal primers (Quan et al., 2005). The PCR product was purified using a QIAquick PCR purification kit (Qiagen). The 16S rRNA gene sequence was determined directly using the PCR-amplified DNA as a sequencing template. A sequencing PCR was performed with forward and reverse primers (Quan et al., 2005) using a DNA Analyzer (3730X1; Applied Biosystems). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the CLUSTAL_X program (Thompson et al., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Phylogenetic trees were constructed on the basis of three tree-making algorithms: neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony

Table 1. Phenotypic characteristics that differentiate strain N4T from other species of the genus Chryseobacterium

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*DNA G+C contents shown are for type strains.
Table 2. Cellular fatty acid profiles (%) of strain N4T and species of the genus Chryseobacterium

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<td>iso-C17:09c</td>
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<tr>
<td>Summed</td>
<td>32.4</td>
<td>11.8±0.8</td>
<td>9.2</td>
<td>11.1±1.3</td>
<td>12.0</td>
<td>11.8±0.6</td>
<td>6.5</td>
<td>12.1±1.3</td>
<td>8.4</td>
<td>6.5</td>
<td>13.8</td>
<td>12.4</td>
<td>9.1±0.9</td>
<td>9.7</td>
<td>11.2</td>
<td>10.8±1.3</td>
<td>26.7</td>
<td>11.0</td>
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</table>

*Summed feature 4 contains iso-C15:0 2-OH and/or C16:1ω7c/t.

(Swofford, 1993) by using the MEGA3 program (Kumar et al., 2004), with bootstrap values based on 1000 replications (Felsenstein, 1985). Evolutionary distances were calculated using the method of Jukes & Cantor (1969).

Details of the cultural, physiological and biochemical characteristics of strain N4T are given in the species description and in Table 1. The results from the API ZYM galleries are available in Supplementary Table S1 in IJSEM Online. The cellular fatty acid profile of strain N4T was characterized by the predominance of summed feature 4 (iso-C15:0 2-OH and/or C16:1ω7c/t), iso-C15:0 and C16:0, and was similar to those of other Chryseobacterium species. However, N4T differed from other Chryseobacterium species (except C. hispanicum) in containing a very large amount of anteiso-C15:0 (Table 2). The predominant respiratory quinone was menaquinone MK-6. The DNA G+C content was 38.2 mol%.

The almost-complete 16S rRNA gene sequence (1483 bp) of strain N4T was determined. The levels of 16S rRNA gene sequence similarity between N4T and the type strains of other Chryseobacterium species ranged from 95.8 % (with C. joostei) to 93.2 % (with C. balustinum). Sequence similarities with all other species of the family Flavobacteriaceae included in the phylogenetic analysis were below 93.4 %. In the phylogenetic tree based on the neighbour-joining algorithm, strain N4T clustered with species of the genus Chryseobacterium (Fig. 1). Similar tree topologies were also found in the trees generated with the maximum-parsimony and minimum-evolution algorithms (data not shown).

The above-mentioned chemotaxonomic features, together with the morphological, physiological and biochemical characteristics, strongly support the classification of strain N4T within the genus Chryseobacterium. The phylogenetic distinctiveness was sufficient to categorize strain N4T as representing a novel species within the genus Chryseobacterium (Stackebrandt & Goebel, 1994). The strain could also be distinguished from other Chryseobacterium species by means of some important phenotypic characteristics (summarized in Table 1): growth conditions, enzyme activities, acid production from carbohydrates, fatty acid profile (Table 2) and API ZYM profile (see Supplementary Table S1 in IJSEM Online). On the basis of the results obtained, strain N4T is sufficiently distinct from all known Chryseobacterium species to be recognized as representing a novel species of the genus Chryseobacterium, for which the name Chryseobacterium caeni sp. nov. is proposed.

Description of Chryseobacterium caeni sp. nov.

Chryseobacterium caeni (ca.e’ni. L. gen. n. caeni of sludge).

Cells are aerobic, non-spore-forming, non-motile rods. Gram-negative, oxidase- and catalase-positive. Good growth is observed on R2A, tryptic soy and nutrient agars, but not on MacConkey agar. Colonies are translucent and shiny with entire edges, becoming mucoid after 3 days.
incubation. Bright yellow flexirubin-type pigments are produced. Growth occurs at 5–37 °C, but not at 42 °C; the optimum temperature for growth is between 28 and 30 °C. The pH range for growth is 6.0–10.0, with an optimum at between pH 6.5 and 8.0. Cells grow in the presence of 0–3 % NaCl, but not with 5 % NaCl. The major fatty acids are summed feature 4 (iso-C15 : 02-OH and/or C16 : 1 ω7c/ω7t), iso-C15 : 0 and C16 : 0. Menaquinone MK-6 is the predominant respiratory quinone. The G+C content of the genomic DNA is 38.2 mol%. Acid is not produced from amygdalin, L-arabinose, D-fructose, D-glucose, glycerol, inositol, lactose, D-melibiose, D-maltose, D-mannitol, L-rhamnose, D-sorbitol, D-sucrose, trehalose or D-xyllose. The following substrates are utilized as sole carbon sources: D-glucose, L-arabinose, D-melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, histidine, 2-ketoglucunate, 3-hydroxybutyrate, 4-hydroxybenzoate, L-proline, L-rhamnose, N-acetylglucosamine, D-ribose, inositol, itaconate, suberate, malonate, acetate, DL-lactate, L-alanine, 5-ketogluconate, 3-hydroxybenzoate, L-serine, D-mannose, gluconate, caprate, adipate, malate or phenylacetic acid. Positive for urease and β-glucosidase activities, but negative for indole production. Nitrate and nitrite are not reduced. Results from the API ZYM test are given in Supplementary Table S1 in IJSEM Online.

The type strain, N4T (=KCTC 12506T =CCBAU 10201T =DSM 17710T), was isolated from the sludge of a nickel-complexed cyanide treatment bioreactor.

**Acknowledgements**

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References


