Reclassification of the members of the genus *Tetrathiobacter* Ghosh *et al.* 2005 to the genus *Advenella* Coenye *et al.* 2005

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The taxonomic position of the genera *Advenella* and *Tetrathiobacter* was examined. 16S rRNA gene sequence analysis revealed that the two genera are closely related, representing a monophyletic cluster with high sequence similarity (98.1–99.7 %) within the family *Alcaligenaceae*. The phenotypic characteristics of the type strains of *Advenella incenata*, *Tetrathiobacter kashmirensis* and *Tetrathiobacter mimigardefordensis* were re-examined using the API 20NE, API ZYM and API 50CH systems. Phylogenetic data together with similarities in phenotypic characteristics, G + C content and cellular acid composition suggest that they should be classified in the same genus. On the basis of the data presented, the two species of the genus *Tetrathiobacter* should be transferred to the genus *Advenella*, since this genus has nomenclatural priority. Therefore, *Tetrathiobacter kashmirensis* and *Tetrathiobacter mimigardefordensis* should be transferred to the genus *Advenella* as *Advenella kashmirensis* comb. nov. (type strain WT001T = LMG 22695T = MTCC7002T) and *Advenella mimigardefordensis* comb. nov. (type strain DPN7T = DSM 17166T = LMG 22922T). Emended descriptions of *Advenella incenata* and the genus *Advenella* are also presented.

In the course of a study of environmental bacteria able to use n-triazines, we isolated several strains from groundwater contaminated by terbutylazine. Preliminary phylogenetic analysis of the 16S rRNA gene sequences of these strains (about 700 nt) revealed that their closest relatives were members of the genera *Advenella* and *Tetrathiobacter*. The genus *Advenella* was proposed by Coenye *et al.* (2005) to accommodate Gram-negative, rod-shaped to cocccoid, oxidase-positive bacteria isolated from various human and veterinary clinical samples. *Advenella incenata* is the type and single species of this genus. The genus *Tetrathiobacter*, with the type species *Tetrathiobacter kashmirensis*, was created by Ghosh *et al.* (2005) to describe Gram-negative, non-flagellated, oval to cocccoid-shaped bacteria occurring singly or in pairs, chains, branched chains or clusters isolated from bulk soils of a temperate orchard in Srinagar, Jammu and Kashmir, India. Another species, *Tetrathiobacter mimigardefordensis*, was described soon after by Wübbeler *et al.* (2006). Both genera are members of the family *Alcaligenaceae* (De Ley *et al.*, 1986). In this study, we present the results of the phenotypic and phylogenetic characterization of the environmental bacterial isolates and a critical taxonomic evaluation of the members of the two genera, and propose the combination of the genus *Tetrathiobacter* with the genus *Advenella*, since the latter has nomenclatural priority.

The environmental isolates 4GA-2008, 6GA-2008 and 7GA-2008 were isolated from groundwater contaminated by terbutylazine, located in Assisi, in central Italy. Primary isolation was achieved on minimal medium (MM) [l-1; 1.6 g K2HPO4, 0.4 g KH2PO4, 0.1 g CaSO4·2H2O, 1 g MgSO4·7H2O, 0.02 g FeSO4·7H2O, 2 g (NH4)2SO4, 15 g agar], supplemented with 2 p.p.m. terbutylazine and 0.03 % Casamino acids, after incubation at 30 °C for 48 h under aerobic conditions. After primary isolation and further subculture on Luria–Bertani (LB) agar plates at 30 °C for 48 h, isolates were stored at −20 °C as glycerol suspensions (20 % v/v). *A. incenata* CCUG 45225T, T.
kashmirensis LMG 22695T and LMG 22696 and T. mimigardefordensis LMG 22922T were obtained from the respective culture collections, grown aerobically at 30°C on LB agar plates for 48 h and stored at −20°C as glycerol suspensions (20% v/v).

Phylogenetic analysis of the environmental isolates was performed by comparative 16S rRNA gene sequence analysis as described previously (Vela et al., 2006). The sequence of a large fragment of the 16S rRNA gene of the three isolates (approx. 1440 bases) as well as that of A. incenata CCUG 45225T (1341 bp; the sequence available previously in GenBank had only 734 nucleotides) was obtained bidirectionally. 16S rRNA gene sequence analysis revealed 100% similarity among the three environmental strains. Sequence searches of GenBank using the program FASTA (Pearson, 1994) confirmed the preliminary sequencing results and confirmed that the environmental isolates were phylogenetically most closely related to T. kashmirensis WT001T (99.7% sequence similarity), A. incenata CCUG 45225T (99.4% to the new, longer sequence) and T. mimigardefordensis DPN7T (99.0%). These sequences and those of other representative members of the family Alcaligenaceae were retrieved from GenBank and aligned with the newly determined sequence by using the program DNATools (Rasmussen, 1995). Phylogenetic trees were constructed according to three different methods, a neighbour-joining algorithm (Saitou & Nei, 1987), performed with the programs DNATools and TreeView (Page, 1996), maximum-likelihood analysis done using the PHYML software (Guindon & Gascuel, 2003) and maximum-parsimony method carried out using the MEGA software package version 3.1 (Kumar et al., 2004). Genetic distances for the neighbour-joining and maximum-likelihood algorithms were calculated by Kimura’s two-parameter model (Kimura, 1980) and close-neighbour interchange (search level = 2, random additions = 100) was applied in maximum-parsimony analysis. The stability of the groupings was estimated by bootstrap analysis (1000 replications). The members of the genera Advenella and Tetrathiobacter formed a monophyletic cluster with 100% bootstrap support and were readily differentiated from other genera of the family Alcaligenaceae (Fig. 1). Within this cluster, T. mimigardefordensis DPN7T formed a distinct subline from that formed by A. incenata CCUG 45225T and T. kashmirensis WT001T with 99% bootstrap support. This tree topology was confirmed by the three phylogenetic algorithms. Pairwise 16S rRNA gene sequence similarity values within this cluster ranged between 98.1 and 99.7%; these values are typical of members of the same genus. The G+C contents of members of the genera Advenella (53.5–58.0 mol%) and Tetrathiobacter (54.0–55.2 mol%) and their cellular fatty acid compositions, with 16:0 and 18:1ω7c as the predominant fatty acids, are very similar (Coenye et al., 2005; Ghosh et al., 2005; Wübbeler et al., 2006).
The phenotypic characteristics described for *A. incenata*, *T. kashmirenensis* and *T. mimigardefordensis* are not directly comparable, because the same characteristics were not determined for the three species (Coenye et al., 2005; Ghosh et al., 2005; Wübbeler et al., 2006). In this study, the environmental isolates were characterized phenotypically and the phenotypic characteristics of *A. incenata* CCUG 45225<sup>T</sup>, *T. kashmirenensis* LMG 22695<sup>T</sup> and *T. mimigardefordensis* LMG 22922<sup>T</sup> were re-examined using commercial kits. Biochemical and enzyme characteristics were determined using the API 20NE and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. Carbohydrate assimilation was assayed by using API 50CH strips (bioMérieux) which were inoculated with a 0.5 McFarland suspension of bacterial cells in AUX medium (bioMérieux). The API 50CH strips were read up to 4 days of incubation at 30 °C. Assimilation of DL-lactate was determined in MM broth containing 1% (w/v) DL-lactate (Sigma). The environmental isolates exhibited almost identical phenotypic characteristics, which matched those exhibited by *A. incenata* CCUG 45225<sup>T</sup> except that they did not hydrolyse urea (*A. incenata* CCUG 45225<sup>T</sup> was positive). *A. incenata* CCUG 45225<sup>T</sup>, *T. kashmirenensis* LMG 22695<sup>T</sup> and *T. mimigardefordensis* LMG 22922<sup>T</sup> also exhibited many common characteristics, although several tests can be used for their differentiation. The results are given in the species descriptions and in Table 1.

The distinct phylogenetic position of *T. mimigardefordensis* and its separate species status with respect to *T. kashmirenensis* were supported by DNA–DNA hybridization experiments and by differences in biochemical and chemotaxonomic characteristics (Wübbeler et al., 2006). Therefore, genomic relatedness was examined to determine the species status of the environmental strains with respect to *A. incenata* and *T. kashmirenensis* and between *A. incenata* and both species of *Tetrathiobacter*. DNA–DNA hybridization experiments were carried out between isolate 4GA-2008 and isolates 6GA-2008 and 7GA-2008, between isolate 4GA-2008 and its nearest phylogenetic neighbours *A. incenata* CCUG 45225<sup>T</sup> and *T. kashmirenensis* LMG 22695<sup>T</sup>, between the two latter strains and between *A. incenata* CCUG 45225<sup>T</sup> and *T. kashmirenensis* LMG 22696 and *T. mimigardefordensis* LMG 22922<sup>T</sup>. DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huß et al. (1983) using a Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multichannel and a temperature controller with *in situ* temperature probe (Varian). Preparation of high-molecular-mass DNA and DNA–DNA hybridization experiments were performed by the DSMZ Identification Service (Braunschweig, Germany). DNA–DNA hybridization between isolate 4GA-2008 and isolates 6GA-2008 and 7GA-2008 showed DNA relatedness values of 95.1 and 84.9%, respectively. The DNA–DNA reassociation values between isolate 4GA-2008 and *A. incenata* CCUG 45225<sup>T</sup>, *T. kashmirenensis* LMG 22695<sup>T</sup> and *T. kashmirenensis* LMG 22696 were 93.9, 44.8 and 31.4%, respectively, demonstrating that the environmental isolates are members of the species *A. incenata* (Wayne et al., 1987). DNA–DNA reassociation values between *A. incenata* CCUG 45225<sup>T</sup> and *T. kashmirenensis* LMG 22695<sup>T</sup>, *T. kashmirenensis* LMG 22696 and *T. mimigardefordensis* LMG 22922<sup>T</sup> were 48.3, 44.0 and 27.7%, respectively. These values are below the recommended threshold value of 70%, confirming that they merit their separate species status (Wayne et al., 1987).

Considering the phenotypic similarities, phylogenetic position and genetic and chemotaxonomic data, *A. incenata*, *T. kashmirenensis* and *T. mimigardefordensis* should be members of the same genus. The names *Advenella* and *Tetrathiobacter* were published in the same year but, according to the Bacteriological Code (Rule 24b), the genus *Advenella* has priority and, consequently, the two species of the genus *Tetrathiobacter* should be reclassified as members of the genus *Advenella*.

Emended description of the genus *Advenella*

*Coenye et al. 2005*

*Advenella* (Ad.ven.el’la. L. n. *advena* a stranger, a foreigner; L. dim. ending -ella; N.L. fem. n. *Advenella* the little stranger, referring to the fact that the source of the first strains was unknown).

The description is as given by Coenye et al. (2005) with the following modifications. Some members of the genus do not assimilate DL-lactate, D-mannose or maltose. The type species is *Advenella incenata*.

Emended description of *Advenella incenata*  

*Coenye et al. 2005*

*Advenella incenata* (in.ce.na’ta. L. fem. adj. *incenata* that has not dined, fasting, referring to the fact that this organism shows little biochemical activity).

### Table 1. Phenotypic characteristics that differentiate type strains of the genus *Advenella*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Assimilation of:</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>D-Fructose</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Melezitose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2-Ketogluconate</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>DL-Lactate</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Esterase (C4) activity</td>
<td>+</td>
<td>–</td>
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The description remains that given by Coenye et al. (2005) with the following additions. Assimilates adipate, glycerol, D- and L-xylene, D-arabinose, galactose, ribose, rhamnose, D- and L-fucose, gluconate and 2-ketogluconate, but does not assimilate erythritol, D-adenonitol, turanose, tagatose, D-lyxose, xylitol, gentiobiose, glycogen, sucrose, melibiose, D- or L-arabitol, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, sorbitol, starch, raffinose, inulin, dulcitol, inositol, L-sorbose, methyl β-D-xylopyranoside, D-fructose, trehalose or melezitose.

The type strain is CCUG 45225T = LMG 22250T, isolated from human sputum in Sweden.

**Description of Advenella kashmirensis comb. nov.**

*Advenella kashmirensis* (kash.mir.en’sis. N.L. masc. adj. kashmirensis of Kashmir, after the name of the province from where the original strains of the species were isolated).


The description is as given for *Tetrathiobacter kashmirensis* by Ghosh et al. (2005) with the following modifications. Assimilates adipate, D- and L-xylene, D-arabinose, galactose, ribose, rhamnose, D- and L-fucose, gluconate and fructose, but does not assimilate erythritol, D-adenonitol, turanose, tagatose, D-lyxose, xylitol, gentiobiose, glycogen, sucrose, melibiose, D- or L-arabitol, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, sorbitol, starch, raffinose, inulin, dulcitol, inositol, L-sorbose, methyl β-D-xylopyranoside, D-fructose, trehalose or melezitose.

The type strain is WT001T (=LMG 22695T = MTCC7002T), isolated from bulk soil of a temperate orchard in Srinagar, Jammu and Kashmir, India.

**Description of Advenella mimigardefordensis comb. nov.**

*Advenella mimigardefordensis* (mi.mi.gar.de.for.den’sis. M.L. masc. adj. mimigardefordensis of Mimegardefordum, a medieval name of Münster, where the type strain was isolated).


The description remains that given for *Tetrathiobacter mimigardefordensis* by Wübberle et al. (2006) with the following additions. Assimilates glycerol, DL-lactate, adipate, D- and L-xylene, D-arabinose, D-galactose, ribose, D- and L-fucose, gluconate and melezitose, but does not assimilate erythritol, D-adenonitol, turanose, tagatose, D-lyxose, xylitol, gentiobiose, glycogen, sucrose, melibiose, starch, D- or L-arabitol, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, sorbitol, raffinose, inulin, dulcitol, inositol, L-sorbose, methyl β-D-xylopyranoside, D-fructose, D-rhamnose, trehalose or 2-ketogluconate. Alkaline phosphatase (weak reaction), acid phosphatase and esterase lipase (C8) are detected. No activity is detected for esterase (C4) or cystine arylamidase.

The type strain is DPN7T (=DSM 17166T = LMG 22922T), isolated from a sample of matured compost from a compost plant in Münster (Germany).

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**References**


