Hephaestia caeni gen. nov., sp. nov., a novel member of the family Sphingomonadaceae isolated from activated sludge

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A Gram-staining-negative, rod-shaped and motile bacterium, designated strain ERB1-3T, was isolated from a laboratory-scale activated sludge system treating coke plant effluent using thiocyanate-supplemented growth medium. Strain ERB1-3T was oxidase-positive and weakly catalase-positive. The predominant fatty acids were C18:1ω7c (35.6%) and C17:1ω6c (29.2%), and the major respiratory quinone was Q-10. Polar lipids were dominated by sphingoglycolipid and phosphatidylglycerol. Major polyamines were spermidine and sym-homospermidine. The G+C content of the genomic DNA of strain ERB1-3T was 66.4 mol%. Based on the 16S rRNA gene, strain ERB1-3T exhibited the highest sequence similarity values to Sphingomonas sanranigenes DSM 19645T (96.1%), Sphingobium scionense DSM 19371T (95.1%) and Stakelama pacifica LMG 24886T (94.8%) within the family Sphingomonadaceae. The novel isolate had some unique chemotaxonomic features that differentiated it from these closely related strains, contained much more C17:1ω6c, C15:0 2-OH, C17:0 and C17:1ω8c fatty acids and possessed diphosphatidylglycerol only in trace amounts. On the basis of the phenotypic, chemotaxonomic and molecular data, strain ERB1-3T is considered to represent a novel genus and species, for which the name Hephaestia caeni gen. nov., sp. nov. is proposed. The type strain is ERB1-3T (=DSM 25527T =NCAIM B 02511T).

A recent study (Felföldi et al., 2010) focusing on the bacterial community of a laboratory-scale activated sludge system removing thiocyanate and phenols from coke plant effluent has revealed some isolates, which showed low pairwise similarity values of partial 16S rRNA gene sequences to those of species with validly published names. One of these strains has been already described as a novel species, Ottowia pentelensis (Felföldi et al., 2011). This paper deals with the detailed taxonomic characterization of another isolate, ERB1-3T, that is proposed to represent a new genus of the family Sphingomonadaceae.

The genus Sphingomonas was originally proposed by Yabuuchi et al. (1990) for Gram-stain-negative, rod-shaped, strictly aerobic, chemoheterotrophic bacteria possessing ubiquinone (Q-10) as the major respiratory quinone and glycosphingolipids in their cell envelopes and forming typically yellow-pigmented colonies (Balkwill et al., 2006). Later, this genus was subdivided into four genera, Sphingomonas sensu stricto, Sphingobium, Novosphingobium and Sphingopyxis (Takeuchi et al., 2001), and in recent years, several additional new genera have been described and assigned to the family Sphingomonadaceae (e.g. Paraphosphopyxis, Sphingosinicella, Sphingomicrobium and Stakelama; Maruyama et al., 2006; Chen et al., 2010; Kämpfer et al., 2012; Uchida et al., 2012). Sphingomonads are widely distributed in nature and have been isolated from different aquatic and terrestrial habitats as well as from clinical specimens (Balkwill et al., 2006). Furthermore, these bacteria are metabolically versatile and are able to utilize a wide range of naturally occurring or refractory organic compounds (Balkwill et al., 2006).

Strain ERB1-3T was isolated from a laboratory-scale activated sludge system treating coke plant effluent at the Eötvös Loránd University (Budapest, Hungary) in 2007 (Felföldi et al., 2010). The standard dilution plating technique was applied to obtain strains from an enrichment culture.
containing sterile reactor filtrate and potassium thiocyanate (50 mg l⁻¹). Strain ERB1-3T was maintained on trypticase soy agar (TSA; Merck) for detailed taxonomic analysis at 28 °C, while authentic strains for side-by-side tests, Sphingomonas sanxianigenes DSM 19645T, Sphingobium scionense DSM 19371T and Stakelama pacifica LMG 24686T (=DSM 25059T), were maintained on TSA, nutrient (medium 1, DSMZ) and seawater agar (Chen et al., 2010), respectively, at the same temperature. Temperature, pH and salt concentration optima were determined in the corresponding media given above. Growth intensity was tested at 4, 20, 25, 28, 37 and 45 °C, at pH 2, 3, 4, 4.5, 5, 6, 7, 8, 8.5, 9 and 10 (adjusted with NaOH and HCl) and with NaCl concentrations of 0, 1.5, 2.5, 5.0 and 7.5 %.

Colony morphology of strain ERB1-3T was tested on TSA medium by direct observation of single colonies. Cell morphology and motility were studied with native preparations and with Gram staining according to the method of Claus (1992). The presence of flagella was demonstrated as described by Heimbrook et al. (1989). Oxidase activity was studied according to the method of Tarrand & Gröschel (1982), catalase, Voges–Proskauer (VP) and methyl red (MR) reaction were performed as described by Cowan & Steel (1974). Acid production from D-glucose was checked by the oxidative and fermentative test according to the protocol of Hugh & Leifson (1953). Urease activity, reduction of nitrate, starch hydrolysis, indole production from tryptophan, hydrolysis of Tween 80 and phosphatase activity were studied with the method described by Mesbah et al. (1991). Oxidase activity was studied according to the method of Claus (1992). The presence of flagella was demonstrated as described by Heimbrook et al. (1989). Oxidase activity was studied according to the method of Tarrand & Gröschel (1982), catalase, Voges–Proskauer (VP) and methyl red (MR) reaction were performed as described by Cowan & Steel (1974). Acid production from D-glucose was checked by the oxidative and fermentative test according to the protocol of Hugh & Leifson (1953). Urease activity, reduction of nitrate, starch hydrolysis, indole production from tryptophan, hydrolysis of Tween 80 and phosphatase activity were studied with the method of Smibert & Krieg (1994). Carbon source utilization and test according to the protocol of Hugh & Leifson (1953).

The 16S rRNA gene of strain ERB1-3T was amplified and sequenced as described by Felföldi et al. (2011). Sequence alignment with closely related strains was performed with SINA (Pruesse et al., 2012). Phylogenetic analysis (including the search for the best fit model) was performed with the MEGA5 software (Tamura et al., 2011).

The 16S rRNA gene sequence determination for strain ERB1-3T resulted in a sequence of 1411 nucleotides. The most closely related species with validly published names were identified using the EzTaxon-e server (Kim et al., 2012) on the basis of the sequence data. The most similar type strain was Sphingomonas sanxianigenes DSM 19645T with pairwise similarity 96.1 %, while all other species of the genus Sphingomonas showed <95.2 % similarity values to ERB1-3T. Sequence similarity values with Sphingobium scionense DSM 19371T (95.1 %) and Stakelama pacifica LMG 24686T (94.8 %) were also close to the threshold value (~95 %) suggested by Tindall et al. (2010) to establish a separate genus. At the same time, phylogenetic analysis based on the 16S rRNA gene showed that ERB1-3T forms a distinct lineage within the members of the family Sphingomonadaceae without any obvious clustering with type strains of related species (Fig. 1). Signature nucleotide analysis also confirmed the distinction of ERB1-3T from members of several closely related genera (Table S1, available in IJSEM Online).

Cells of strain ERB1-3T were Gram-stain-negative, motile by means of a polar flagellum, aerobic and mesophilic with a characteristic heterotrophic metabolism (Table 1; Fig. S1). Detected quinones of ERB1-3T were Q-10, Q-11, Q-8 and Q-9 at a ratio of 84 : 13 : 1 : 1, which confirmed the position of the novel strain within the family Sphingomonadaceae, since ubiquinone Q-10 is the major respiratory quinone of sphingomonads (Busse et al., 1999; Balkwill et al., 2006). The fatty acid pattern of strain ERB1-3T was dominated by C18:1ω7c and C17:0ω6c, while C15:0 2-OH, C17:0, C14:0 2-OH, C17:0ω8c, C16:0 0cyclo-C19:0ω9c, C15:0 and C18:1ω9c were present as minor components (<10 %) (Table 2). Since fatty acid profile analysis has played a central role in the pioneering taxonomic studies of the family Sphingomonadaceae (Busse et al., 1999; Takeuchi et al., 2001; Yabuuchi et al., 2002), besides the most closely related type strains shown in Table 1, all type species of closely related genera were involved in our comparative analysis (Table 2). In members of the family Sphingomonadaceae, C18:1ω7c is the characteristic predominant fatty acid and the presence of 2-hydroxy fatty acids, mainly C14:0 2-OH, is common, while C17:0ω6c is usually present in lower amounts or is even absent (Busse et al., 1977) and subsequently degraded into nucleosides by P1 nuclease and bovine intestinal mucosa alkaline phosphatase as described by Moshah et al. (1989). The nucleosides were separated by reversed–phase HPLC (LC 20A; Shimadzu) as described by Tamaoka & Komagata (1984). The G+C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine by using standard DNAs with published genome sequences.

The DNA for analysis of the base composition was isolated from cells disrupted with a French press. DNA was purified on hydroxyapatite according to the protocol of Cashion et al. (1977) and subsequently degraded into nucleosides by P1 nuclease and bovine intestinal mucosa alkaline phosphatase as described by Moshah et al. (1989). The nucleosides were separated by reversed–phase HPLC (LC 20A; Shimadzu) as described by Tamaoka & Komagata (1984). The G+C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine by using standard DNAs with published genome sequences.

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The polar lipid pattern of strain ERB1-3\(^T\) contained the closely related type strains (Table 2).

2009; Chen

1999; Takeuchi

T. Felföldi and others

the most closely related type strains, and in ERB1-3\(^T\), C\(_{15}:0\) 2-

Sphingomonas sanxanigenens

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\(\text{OH} \) was another characteristic hydroxy fatty acid (representing

f o u n dh i g h a m o u n t s i n E R B 1 - 3 T (29.2 \%) compared with

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Table 1. Phenotypic and biochemical characteristics of ERB1-3\(^T\) and related type strains

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<td>Motility</td>
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<td>Oxidase</td>
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<td>Catalase</td>
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<td>Fermentative acid production from D-glucose</td>
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<td>Starch hydrolysis</td>
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<td>(\text{NO}_3^-) → (\text{NO}_2^-)</td>
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<td>(\text{NO}_3^-) → (\text{NH}_3)</td>
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<tr>
<td>Salt tolerance (NaCl)</td>
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<td>0–7.5 %</td>
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<td>pH tolerance</td>
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<td>5.0–8.5</td>
<td>3.0–9.0</td>
<td>5.0–9.0</td>
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<td>Growth at 4 (^\circ)C</td>
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<td>Tween 80 hydrolysis</td>
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<td>Assimilation of (API 50 CH)</td>
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<td>L-Rhamnose</td>
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<td>D-Mannitol</td>
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<td>Enzyme activity (API ZYM)</td>
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<td>Valine arylamidase</td>
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<td>Trypsin</td>
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<td>(\alpha)-Chymotrypsin</td>
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<td>(N)-Acetyl-(\beta)-glucosaminidase</td>
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<td>DNA G + C content (mol%)</td>
<td>66.4</td>
<td>66.4*</td>
<td>63.8*</td>
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*Data from Huang et al. (2009), Liang & Lloyd-Jones (2010) and Chen et al. (2010).
and chemotaxonomic investigations of strain ERB1-3T are given in the genus and species descriptions below and in Table 1.

Based on the comparative data presented in this study, strain ERB1-3T is considered to represent a novel genus and species within the family Sphingomonadaceae, for which the name Hephaestia caeni gen. nov., sp. nov. is proposed.

**Description of Hephaestia gen. nov.**

Hephaestia [Heph.aes’ti.a N.L. fem. n. Hephaestia belonging to Hephaestus; named after Hephaestus (Hephaistos), the god of fire and metals, the protector of blacksmiths and craftsmen in Greek mythology; and refers to the fact that the type strain was isolated from an activated sludge system treating the coke plant effluent of a steelworks].

Cells are Gram-stain-negative motile rods. Aerobic and mesophilic. Oxidase- and (weakly) catalase-positive. The major respiratory quinone is ubiquinone (Q-10). The major cellular fatty acids are C₁₈:₁₀₋₇c and C₁₇:₁₀₋₆c. Polar lipids are dominated by sphingoglycolipid and phosphatidylglycerol. Major polyamines are spermidine and *syn*-homospermidine. The cell wall contains *meso*-diaminopimelic acid.

The type species is *Hephaestia caeni.*

**Description of Hephaestia caeni sp. nov.**

*Hephaestia caeni* (cae’nī. L. gen. n. caeni of mud, referring to that the type strain was isolated from activated sludge). Cells are rod-shaped (0.3–0.7 × 1.0–2.9 μm) and motile. Colonies on TSA medium are beige-coloured, circular, raised, shiny and 1–2 mm in diameter. Growth occurs at 20–37 °C (optimum, 28 °C), at pH 5–8.5 (optimum, pH 6–8) and 0–1.5 % NaCl. Negative for the reduction of nitrate to nitrite or ammonia, not capable for denitrification. Positive for phosphatase activity, starch and Tween 80 hydrolysis. Negative for VP and MR reaction, oxidative and fermentative acid production from D-glucose, urease activity and indole production. Positive for assimilation of aesculin and D-fucose and negative for glycerol, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-rhamnose, D-mannitol, methyl D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, sucrose, trehalose,
melezitose, raffinose, starch, gentiobiose, turanose, erythritol, d-arabinose, d-ribose, l-xylose, d-adenitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, d-sorbitol, methyl α-D-mannopyranoside, lactose, melibiose, inulin, glycogen, xylitol, d-lyxose, d-tagatose, L-fucose, d-arabitol, L-arabitol, glucionate, 2-ketogluconate, 5-ketogluconate. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase, esterase lipase (C8), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase and negative for lipase (C14), α-mannosidase and α-fucosidase enzyme activities. The major hydroxy fatty acids are C14:0 2-OH and C15:0 2-OH.

The type strain, ERB1-3T (=DSM 25527T=NACAIM B 02511T), was isolated from the activated sludge of a laboratory-scale model system treating coke plant effluent. The G+C content of the genomic DNA is 66.4 mol%.

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