Emended description of the species *Lampropedia hyalina*

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Three *Lampropedia hyalina* strains from different habitats were compared by phenotypic, chemotaxonomic and molecular characteristics. All strains form coccoid cells and have been reported to grow as square tablets of eight to 64 cells. However, two of these strains (ATCC 11041^T and ATCC 43383) have apparently lost this ability, and the third strain may temporarily lose this capacity under certain cultivation conditions. The three strains showed only minor differences in metabolic characteristics: the main significant physiological difference was the ability to accumulate polyphosphate under alternating anaerobic–aerobic conditions found for DSM 15336. The three strains showed high similarity in fatty acid composition and only slight differences in the G+C content (63–67 mol%) and DNA–DNA reassociation (90–95 % relatedness). Comparative 16S rRNA gene sequence analyses on these three strains and three *Lampropedia hyalina* 16S rRNA gene sequences deposited at NCBI showed that they are all very similar (>98·8 %) and that they form a distinct group among the '*Betaproteobacteria*', showing between 94·6 and 93 % 16S rRNA gene similarity to members of various genera such as *Acidovorax*, *Aquaspirillum*, *Brachymonas*, *Comamonas*, *Delftia* and *Xenophilus*. Fluorescent *in situ* hybridization with oligonucleotide probes targeting betaproteobacteria on the 16S rRNA and 23S rRNA gene level further supported the conclusion that all investigated strains are members of the '*Betaproteobacteria*'. Two oligonucleotide probes were designed and successfully applied for culture-independent identification of *Lampropedia hyalina* by means of fluorescent *in situ* hybridization.

A specimen of the square-tablet-forming, coccoid, heterotrophic genus *Lampropedia* was described for the first time in 1886 by Schroeter in polluted water samples (Schroeter, 1886). Since then, cells similar to Schroeter's description have been observed on several occasions in aquatic systems rich in organic material, rumen or the intestinal system in some animals (Murray, 1984). Our knowledge of this genus is limited, since it is based mainly on the phenotypic characteristics of one species, *Lampropedia hyalina*, which is represented by four strains deposited at the ATCC. However, this collection demands further investigation, since the type strain ATCC 11041^T has apparently lost the tablet-forming ability and grows instead as single coccoid cells. Murray (1984) thus stated that a neotype strain might have to be declared. Recently, Stante *et al.* (1997) isolated a tablet-forming *Lampropedia*-like specimen (DSM 15336) from activated sludge laboratory plants treating dairy and piggery wastewater that were operated as an enhanced biological phosphorus-removing system. This strain differs in at least one metabolic aspect from the *Lampropedia hyalina* type strain, i.e. the ability to accumulate polyphosphate under alternating aerobic–anaerobic conditions (Stante *et al.*, 1997). The aim of our study was to compare the phenotypic, chemotaxonomic and molecular characteristics of this isolate with two *Lampropedia hyalina* strains

Published online ahead of print on 23 March 2004 as DOI 10.1099/ijs.0.02885-0.

Abbreviation: GTA, green top agar.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Lampropedia hyalina* strains DSM 15336, ATCC 11041^T and ATCC 43383 are respectively AY291119, AY291120 and AY291121.

from the ATCC isolated from other environments and to design specific oligonucleotide probes for fluorescent *in situ* hybridization for culture-independent identification of *Lampropedia hyalina*.

The following strains were used: *Lampropedia hyalina* ATCC 11041^T (=CCUG 48573^T=DSM 16112^T), isolated in 1966 by E. G. Pringsheim from liquid manure from a dairy farm yard; *Lampropedia hyalina* ATCC 43383 (=CCUG 48574), isolated by J. Kirchner from the rumen of a fistulated heifer (Hungate, 1966); and *Lampropedia* sp. DSM 15336 (=CCUG 48575), isolated by Stante *et al.* (1997) from activated sludge. For standard cultivations, all three strains were grown at pH 7·2 and at 25 °C on a medium containing moderate amounts of peptone, acetate, yeast extract and soil extract [ATCC culture medium 32; green top agar (GTA)] or on a modified version of this medium made by replacing the soil extract with filtered and sterilized effluent from a diary wastewater treatment plant (Stante *et al.*, 1997). Fresh and stained cells (Gram, Neisser, Sudan black B and Nile blue A) were viewed by light and fluorescence microscopy, respectively, as described by Stante *et al.* (1997). All three strains are unpigmented and produce white colonies with internal refractile structures on GTA plates at 25 °C. Strains ATCC 11041^T and ATCC 43383 have distinctive, round colonies, whereas strain DSM 15336 produces round colonies with partly square borders. Strain ATCC 43383 produces slimy, glossy, transparent colonies, whereas strains ATCC 11041^T and DSM 15336 produce dry, matt, transparent colonies. Growth was usually obtained after 1–3 days at 25 °C. All three investigated strains have been reported to be Gram-negative, coccoid cells (1·0–1·5 × 1·0–2·5 μm), able to produce square tablets of eight to 64 cells (Murray, 1984; Stante *et al.*, 1997). However, both ATCC strains have apparently lost this ability, despite strict cultivation under the conditions recommended by the initial discoverers. Strain ATCC 11041^T was already reported to have lost this ability in 1963 (Murray, 1963), whereas our study is the first to report that ATCC 43383 has also lost this ability. Strain DSM 15336 is still producing square tablets when cultivated on GTA medium, on plates or broth, at neutral pH and at temperatures up to 30 °C (Fig. 1). However, strain DSM 15336 may also lose the tablet-forming ability and produce coccoid cells, in some cases even abnormally enlarged (up to 3·5 μm), under certain conditions, such as when grown on GTA plates or in broth at 37 °C. Interestingly, an identical observation was also reported for ATCC 11041^T when it was still able to produce tablet structures (Lany, 1972). A similar loss of the tablet morphology of strain DSM 15336 could also be induced by growth at 30 °C on either a modified GTA medium (plates or broth) in which acetate was replaced by sucrose (GTASuc) or on Luria–Bertani (LB) medium (plates or broth). No such effect was observed when the cells were grown on GTA plates or broth at pH 9. Hybridization of cells of strain DSM 15336 cultivated on GTASuc plates or on LB plates at 30 °C or on GTA plates at 37 °C with a fluorescent oligonucleotide probe that targeted *Lampropedia hyalina* specifically (probe LAMP444; described below) confirmed that all cells were *Lampropedia hyalina*. Furthermore, when cells of strain DSM 15336 were reinoculated onto GTA plates after cultivation on GTA plates at 37 °C or on GTASuc plates as well as on LB plates cultivated at 30 °C and cultivated at 30 °C, they once again produced square tablets, thus indicating clearly that the tablet-forming ability is dependent on the cultivation conditions.

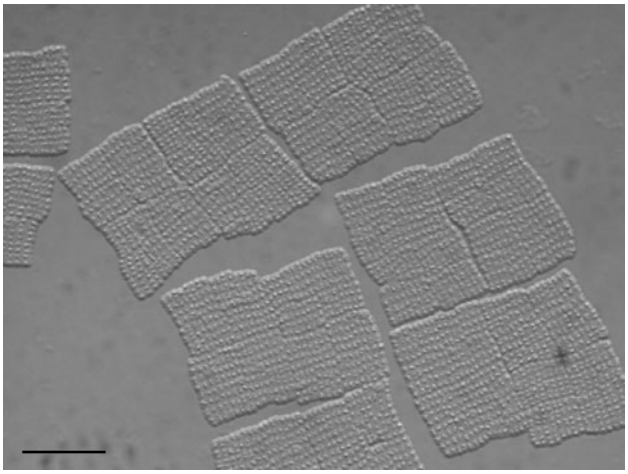

Fig. 1. Bright-field micrograph of cells of *Lampropedia hyalina* DSM 15336. Bar, 10 μm.

The generation times for strains ATCC 11041^T, ATCC 43383 and DSM 15336 were 27·8, 22·6 and 13·3 h, respectively, based on the measurement of cell density by spectrophotometry (Thermo Spectronic Helyos α) at 620 nm on cells cultivated in triplicate on GTA broth at pH 7·2. Tolerance tests were performed on GTA plates in triplicate at different temperatures (10, 20, 30, 40 and 50 °C), pH values (3, 5, 6, 7, 8 and 9) and sodium chloride concentrations (1, 1·5 and 2 %). Bile tolerance was determined by addition of 0·5 % bile to GTA medium (plates). Sensitivity against kanamycin (50 mg l^{-1}) was investigated on GTA plates. Production of N_2 under anoxic conditions was examined as described by Gerhardt *et al.* (1994). Cultivation of cells under cyclic anaerobic–aerobic conditions for

†This strain has also previously been described to be able to form square tablets of 16 to 64 cells (Austin & Murray, 1990); however, in this study (also reported by the ATCC, from which it was retrieved), it seems to have lost this ability.

‡Coccoid cells may be produced when grown on GTA medium (plates or broth) at 37 °C or on other media (at 30 °C) such as LB or on GTA medium (plates or broth) where acetate has been replaced by sucrose (GTASuc).

§Data from other studies are indicated by: *a*, Murray (1984); *b*, Stante *et al.* (1997); *c*, Pringsheim (1966); *d*, Hungate (1966). All other data from this study.

Table 1. Summary of differences between three *Lampropedia hyalina* strains

All three strains showed similar reactions in the following tests. Negative reactions: Gram-stain; anaerobic growth; nitrate reduction; litmus milk; kanamycin tolerance (50 mg l^{-1}); acid production from 21 sugar compounds (adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, glucose, inositol, lactose, maltose, D-mannose, D-mannitol, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, D-xylose); production of *N*-acylhomoserine lactone (AHL, PKRC12, PASC8, PSB403); hydrolysis of aesculin, gelatin, *p*-nitrophenyl (pNP) *β*-D-galactopyranoside, pNP *β*-D-glucuronide, pNP α-D-glucopyranoside, pNP *β*-D-glucopyranoside, pNP *β*-D-xylopyranoside, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxythymidine-5′-pNP phosphate, L-alanine *p*-nitroanilide (pNA), L-glutamate-*γ*-3-carboxy pNA, L-proline pNA, starch and urea; and assimilation of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α-D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose; adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol; *cis*-aconitate, *trans*-aconitate, adipate, azelate, citrate, itaconate, mesaconate, oxoglutarate, suberate; L-alanine, *β*-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. Positive reactions: growth on GTA medium, LB medium and R2A medium; lipid inclusions (analysed by the Sudan black stain); catalase; oxidase; NaCl tolerance 1·5 %; pH tolerance 6–9; temperature tolerance 10–40 °C; and assimilation of acetate, DL-3-hydroxybutyrate and propionate. ND, Not determined.

Characteristic	ATCC 11041^T	ATCC 43383	DSM 15336
Properties on GTA plates:			
Cellular morphology	Single coccoid cells, 1·0–1·5 μm, occasionally arranged as diplococci or tetrads*	Single or diplococcus-like cells, 1·5–2·0 μm†	Coccoid cells, 1·5–2·0 μm, arranged in square sheets of eight to 64 cells‡
Motility	Twitching movements may occur[a]§	Twitching movements may occur[a]	−[b]
Colony morphology and appearance	Thin, hydrophobic surface; circular with regular borders; colour white.[a] Dry, matt and transparent	Circular with regular borders; colour white.[a] Slimy, glossy and transparent	Thin, hydrophobic surface; circular, partly square borders; colour white.[b] Dry, matt and transparent
Isolation source	Polluted aquatic sample[c]	Rumen, fistulated heifer[d]	Activated sludge[b]
Neisser staining (polyphosphate)	−	−	+[b]
Uptake and release of phosphate	−	−	+[b]
Growth in the presence of 0·5 % bile	+ (−)[a]	−	−
Final pH in culture medium	8·4–8·6[a]	8·3–8·5	8·3–8·5
Generation time (GTA broth, 28 °C)	27·8±1·0	22·6±2·01	13·3±1·7
Parasite of warm-blooded hosts	−[a]	ND	ND
Pathogenic for man	−[a]	ND	ND
Hydrolysis of bis-pNP phosphate	−	+	−
Assimilation of:			
L-Aspartate	+	−	−
4-Aminobutyrate	+	−	+
Fumarate	+	−	+
Glutarate	+	−	−
4-Hydroxybenzoate	+	−	−
DL-Lactate	+	−	+
L-Malate	+	−	+
Putrescine	+	−	−
Pyruvate	+	−	+
Fatty acid composition (%):			
$C_{11:0}$ anteiso	−	−	0·1
$C_{10:0}$ 3-OH	6	5·8	6
$C_{12:0}$ 3-OH	2	2·3	2·3
$C_{14:0}$	9·1	9·7	10·2
$C_{16:0}$	24·4	26·2	25·7
$C_{18:1}\omega 7c$	25·9	25·9	24·2
Total sum	100	99·7	99·3
DNA G+C content (mol%)	60·7–61·2[a]; 63·2±0·1	66·7±0·35	64·4±0·40

*Original description: sheets of rounded, almost cubical cells, arranged in square tablets of 16 to 64 cells. However, this strain has mutated and grows now only as single or clustered coccoid cells (Murray, 1963).

stimulation of polyphosphate accumulation was performed as described by Stante *et al.* (1997). Two other standard media, LB plates and R2A plates (Reasoner & Geldreich, 1985), were also used to test whether these media supported growth of *Lampropedia*. All tests were followed for up to 7 days. Tests for oxidase, catalase and litmus milk were performed as described by Gerhardt *et al.* (1994). Metabolic fingerprinting (Table 1) was performed by using the miniaturized assay method described by Kämpfer *et al.* (1991). Hydrolysis of gelatin, starch and urea were examined as described by Gerhardt *et al.* (1994).

In summary, the three *Lampropedia* strains were strongly similar in terms of general phenotypic characteristics (Table 1). The major differences observed among the three strains were in the ability to accumulate polyphosphate under anaerobic–aerobic cyclic incubation conditions (Stante *et al.*, 1996, 1997), bile tolerance (albeit weakly), the ability to hydrolyse one compound (out of 16 tested), bis-*p*-nitrophenyl phosphate, and some minor differences in assimilation of different substrates. Furthermore, growth measurements on GTA broth (containing acetate, one of the three compounds that could be assimilated by all three strains; Table 1) showed that the rate of utilization of acetate [analysed as acetic acid by GC (DANI 8510) as described by Stante *et al.*, 1997] differed significantly between the three strains. DSM 15336 showed the highest rate of utilization (results not shown).

The composition of fatty acids was determined as described by Kämpfer *et al.* (2003) on cells grown on nutrient agar plates with 1 % (w/v) sodium acetate and on GTA plates at 28 °C. The three strains showed nearly identical fatty acid compositions. The main fatty acid methyl ester components detected were $C_{10:0}$ 3-OH (5·8–6 %), $C_{12:0}$ 3-OH (2–2·3 %), $C_{14:0}$ (9·1–10·7 %), $C_{16:0}$ (24·4–26·2 %) and $C_{18:1}\omega7c$ (24·2–25·9 %). The presence of C_{10} 3-OH and C_{12} 3-OH has also been reported for other members of the *Comamonadaceae* (Wen *et al.*, 1999; Spring *et al.*, 2004).

Analysis of the production by the *Lampropedia* strains of three different quorum-sensing *N*-acylhomoserine lactone molecules, PKRC12, PASC8 and PSB404, representative for some betaproteobacteria, was performed as described by Steidle *et al.* (2002). However, none of the strains produced any of the *N*-acylhomoserine lactone molecules investigated (Steidle *et al.*, 2002).

Genomic DNA was extracted and purified for further molecular analyses, as described by Ziemke *et al.* (1998). For subsequent sequencing of the 16S rRNA gene, almost complete 16S rRNA gene fragments were amplified from DNA by using the primers 27F and 1492R (Lane, 1991). For 16S rRNA gene amplification, reaction mixtures (total volume, 50 µl) containing 20 ng DNA template and each primer at a concentration of 25 pM were prepared by adding 20 mM $MgCl_2$ reaction buffer (Idaho Technology) and 2·5 U *Taq* DNA polymerase (Promega). Thermal cycling was carried out by using an initial denaturation step of 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 50 °C for 40 s and elongation at 72 °C for 90 s. Cycling was completed by a final elongation step of 72 °C for 10 min. Prior to sequencing, PCR products were purified by means of the QIAquick PCR purification kit (Qiagen). Nucleotide sequences were determined by the sequencing protocol from Epicenter Technologies (Biozyme). The new 16S rRNA gene sequences (>1400 nt, <0·5 % ambiguity) were added to an alignment of about 50 000 small-subunit rRNA sequences by using the alignment tool of the ARB program package (Ludwig *et al.*, 2004). Alignments were refined by visual inspection. Phylogenetic analyses were performed by using distance-matrix, parsimony and maximum-likelihood (AXML in the ARB software) methods. Various datasets comparing different selections of sequences and alignment positions were used (Ludwig *et al.*, 1998). The three *Lampropedia* strains used in this study showed strong similarity (98·8–99·8 %) to each other at the 16S rRNA gene level. Further sequence comparison with three additional *Lampropedia hyalina* 16S rRNA gene sequences available on the NCBI public database (http://www.ncbi.nlm.nih.gov/; accession numbers AB086632, AB092691 and AB089485), previously placed within the '*Gammaproteobacteria*' (*Pseudomonadaceae*) (Bergey's taxonomic outline, July 2002) but recently moved to the '*Betaproteobacteria*' (Bergey's taxonomic outline, October 2003; Xie *et al.*, 2003), showed strong similarity to the strains in this study (e.g. 99·8 % 16S rRNA gene similarity to the type strain ATCC 11041^T). All *Lampropedia hyalina* strains form a distinct group among the '*Betaproteobacteria*', showing 94·6–93 % 16S rRNA gene sequence similarity to members of various genera such as *Acidovorax*, *Aquaspirillum*, *Brachymonas*, *Comamonas*, *Delftia* and *Xenophilus*. The closest cultured relative is most likely *Brachymonas denitrificans* (94·6 % 16S rRNA gene sequence similarity), as suggested by distance matrix, parsimony and maximum-likelihood treeing methods (Fig. 2).

Determination of the G+C content and DNA–DNA reassociation experiments were carried out as described by Ziemke *et al.* (1998). The G+C contents of strains ATCC 11041^T, ATCC 43383 and DSM 15336 were respectively 63·2 mol% [slightly higher in comparison with the preliminary result of around 60 mol% cited by Murray (1984)], 66·7 and 64·4 mol%. DNA–DNA reassociation studies showed a relatively higher reassociation between DSM 15336 and ATCC 11041^T (94·7 %, SD 0·6 %) than between ATCC 11041^T and ATCC 43383 (90·4 %, SD 0·6 %). These data indicate clearly that all three strains can be classified within the same species, *Lampropedia hyalina*.

For culture-independent identification of *Lampropedia hyalina* by means of fluorescent *in situ* hybridization, cells were fixed in exponential phase in 4 % (w/v) paraformaldehyde for 3 h at 4 °C, as described by Amann (1995). *In situ* hybridization of the samples with oligonucleotides labelled with the monofunctional, hydrophilic, sulfoindocyanine

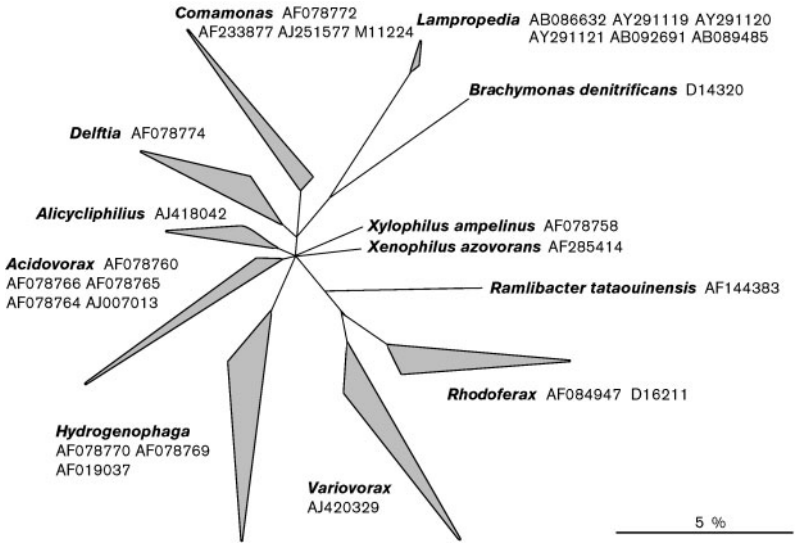

Fig. 2. 16S rRNA gene-based tree reflecting the relationships of *Lampropedia hyalina* and selected representatives of the *Comamonadaceae*. The tree topology is based upon the results of a maximum-parsimony analysis (ARB_parsimony; Ludwig *et al.*, 2004) of 28 277 at-least 90 % complete small-subunit rRNA gene primary structures and was corrected according to data obtained by applying alternative treeing approaches. Alignment positions variable in more that 50 % of all available 16S rRNA gene sequences from representatives of the *Comamonadaceae* were excluded from the calculations. Multifurcations indicate that a common stable branching order was not significantly supported applying different treeing methods on various datasets. Phylogenetic groups are indicated by triangles. Accession numbers are given for the respective type strains. Bar, 5 % estimated sequence divergence.

dye Cy3 (purchased from Interactiva) was performed as described by Amann (1995). Samples subjected to fluorescent *in situ* hybridization were mounted with Citifluor and viewed using a model LSM 510 scanning confocal microscope (Carl Zeiss) equipped with a UV laser (351 and 364 nm), an Ar ion laser (450 to 514 nm) and two HeNe lasers (543 and 633 nm). Different probes were used for identification of *Lampropedia hyalina*. For confirmation of the phylogenetic position among the '*Betaproteobacteria*', two general probes targeting the betaproteobacteria on the 23S rRNA gene level (BET42a; Manz *et al.*, 1992) and on the 16S rRNA gene level (BONE, together with the competitor for BTWO; Amann *et al.*, 1996) were used. All three investigated *Lampropedia hyalina* strains yielded bright signals with these probes. Probes that target *Lampropedia hyalina* specifically were also developed. Probe design was performed on the basis of 50 000 complete and partial 16S rRNA gene sequences present in the ARB database (http://www.arb-home.de) and updated (January 2004) with all relevant existing sequences of *Lampropedia hyalina* and close relatives. Three specific probes were selected on the basis of the 16S rRNA gene sequence: LAMP114 (5′-CGTTCCGATGTCTTACTC-3′), LAMP444 (5′-CCCAGTCCTTTTCGTTCT-3′) and LAMP1017 (5′-TCTTTCGAGCACAATCCC-3′). Optimization of the hybridization conditions for these probes was performed by hybridizing at different formamide concentrations, ranging from 0 to 60 %, and determining the optimal signal intensity visually by microscopy (quantitative single-cell signal intensity measurement was not possible due to the tablet clusters of DSM 15336). The *Lampropedia* strains investigated in this study showed no autofluorescence and showed significant hybridization signals with probes LAMP114 and LAMP444 in the absence of formamide. No signals were obtained with probe LAMP1017 in the absence of formamide, which indicates that this target region is inaccessible. Different optimal hybridization conditions were observed for probes LAMP114 and LAMP444 for the three *Lampropedia* strains. Probe LAMP114 yielded strong hybridization signals at a formamide concentration up to 10 % with the two strains ATCC 11041^T and ATCC 43383 and up to 30 % with DSM 15336. Probe LAMP444 yielded strong hybridization signals at a formamide concentration up to 40 % with all three strains. Hybridization with probes LAMP114 and LAMP444 with members of two distantly related genera among the '*Betaproteobacteria*', *Comamonas testosteroni* and *Hydrogenophaga flava*, in the absence of formamide yielded no signals.

Considerations regarding the taxonomy of *Lampropedia hyalina* ATCC 11041^T

This study shows clearly that the two investigated strains of *Lampropedia hyalina* from the ATCC (ATCC 11041^T and ATCC 43383) and *Lampropedia* sp. DSM 15336 are strongly similar in terms of general physiological, chemotaxonomic and molecular characteristics (Table 1), despite differences in morphology and isolation source. They thus belong to the same species, according to the directions of Stackebrandt *et al.* (2002). The tablet morphology should not be decisive for the species description and thus is also not an argument for the declaration of a neotype strain for *Lampropedia hyalina*, as suggested by Murray (1984). Tablet morphology is unique in nature, but has nevertheless been observed in two other genera, representing entirely different physiological lifestyles: the anaerobic, sulfur-oxidizing *Thiopedia* [16S rRNA gene phylogenetic position unknown (Pfennig, 1989); possibly '*Gammaproteobacteria*' (Bergey's taxonomic outline, October 2003)] and the photosynthetic *Merismopedia* (cyanobacteria), which has also been reported

to lose its tablet morphology under certain conditions (Palinska *et al.*, 1996). Furthermore, the ability of DSM 15336 to accumulate polyphosphate, under cyclic aerobic–anaerobic conditions, in contrast to the other two investigated strains, does not present a strong enough argument for the declaration of a distinct subspecies. For example, polyphosphate-accumulating *Acinetobacter* strains have also been isolated from activated sludge but, despite thorough taxonomic comparison of these strains with non-polyphosphate-accumulating *Acinetobacter* strains, a further subspecies division of these strains could not be made on the basis of this capacity (Kämpfer *et al.*, 1992). On the basis of the results generated in this study, emended descriptions of the genus *Lampropedia* and the species *Lampropedia hyalina* are presented.

Emended description of the genus *Lampropedia* Schroeter 1886

Based on the properties given in the genus description by Murray (1984), members of the genus *Lampropedia* are coccoid species, able under certain conditions to form square tablets of eight to 64 cells, occasionally separated into pairs or tetrads. Their DNA G+C content is between 63 and 67 mol% and their fatty acid profile consists of $C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH, $C_{14:0}$, $C_{16:0}$ and $C_{18:1}\omega7c$. Assimilation of acetate, DL-3-hydroxybutyrate and propionate. Closest cultured relative is *Brachymonas denitrificans* (94·6 % 16S rRNA gene similarity). The type species is *Lampropedia hyalina*.

Emended description of *Lampropedia hyalina* (Ehrenberg 1832) Schroeter 1886

In addition to the properties given in the genus description above, members of the species may share some minor additional phenotypic characteristics listed in Table 1. To date, only three strains of *Lampropedia hyalina* have been characterized: the type strain (ATCC 11041^T = CCUG 48573^T = DSM 16112^T), isolated from polluted water (Pringsheim, 1966); strain ATCC 43383 (=CCUG 48574), isolated from the rumen of a fistulated heifer (Hungate, 1966); and strain DSM 15336 (=CCUG 48575), isolated from activated sludge (Stante *et al.*, 1997). The type strain and strain ATCC 43383 have apparently lost the ability to form tablet structures, whereas DSM 15336 is still able to form tablet structures. Despite this difference in morphology, their different isolation sources and some minor phenotypic differences (Table 1), all three strains show high DNA–DNA reassociation (90–95 %) and high 16S rRNA gene sequence similarity (>98 %).

The type strain is strain ATCC 11041^T (=CCUG 48573^T = DSM 16112^T).

Acknowledgements

The following colleagues are acknowledged: Dr F. Malaspina and Dr S. Gemelli for a precious collaboration; Dr A. Tilche, European Commission (Key Action 'Sustainable Management and Quality of Water'), Dr G. Bortone, Servizio Tutela e Risanamento Risorsa Acqua, Regione Emilia Romagna, and Dr M. Wagner, University of Vienna, for encouragement of this work; Dr K. Riedel and Dr A. Steidle, Lehrstuhl für Mikrobiologie, Technische Universität München, for analysis of *N*-acylhomoserine lactone signal molecules; and M. Valens, Grup d'Oceanografia Interdisciplinari Institut Mediterrani d'Estudis Avançats, Spain, for technical assistance.

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