Reclassification of *Pseudomonas mephitica* Claydon and Hammer 1939 as a later heterotypic synonym of *Janthinobacterium lividum* (Eisenberg 1891) De Ley *et al.* 1978

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Pseudomonas mephitica CCUG 2513^T has been reinvestigated to clarify its taxonomic position. 16S rRNA gene sequence comparisons demonstrated that this strain clusters phylogenetically closely with *Janthinobacterium lividum* (99.8 % sequence similarity to the type strain). Investigation of fatty acid patterns, polar lipid profiles, polyamine patterns and quinone systems supported this delineation. Substrate utilization profiles and biochemical characteristics displayed no differences from the type strain of *J. lividum*, CCUG 2344^T. Therefore, the reclassification of *Pseudomonas mephitica* as a later heterotypic synonym of *Janthinobacterium lividum* is proposed, based upon the estimated phylogenetic position derived from 16S rRNA gene sequence data and chemotaxonomic and biochemical data.

Pseudomonas mephitica was initially proposed as the name for a bacterial strain isolated from butter (Claydon & Hammer, 1939). The species description was based on morphological characteristics, i.e. a Gram-negative bacillus with polar flagellation, as well as physiological traits. The species name was included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980).

Previously, it was recognized that *P. mephitica* is phylogenetically related to *Janthinobacterium* and related organisms (Anzai *et al.*, 2000). The current definition of the genus *Janthinobacterium* is based on the descriptions of De Ley *et al.* (1978) and Sneath (1984) with the emendations of Lincoln *et al.* (1999) and Gillis & Logan (2005). Most *Janthinobacterium lividum* strains produce violacein, but non-pigmented colonies are often encountered (Gillis & Logan, 2005). *P. mephitica* did not produce pigmented colonies. Lincoln *et al.* (1999) pointed out that the fatty acid pattern serves to differentiate the genus *Janthinobacterium* from members of other genera showing a high degree (95 %) of 16S rRNA gene sequence similarity. Q-8 is the major

respiratory lipoquinone, as in all members of the *Betaproteobacteria* studied to date (Yokota *et al.*, 1992). The major phospholipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The fatty acid composition comprises C_{10:0} 3-OH, C_{12:0}, C_{12:0} 2-OH, C_{14:0}, C_{16:0}, C_{16:1} ω7c, C_{17:0} cyclo and C_{18:1} ω7c. The polyamine pattern, with the major compounds 2-hydroxyputrescine and putrescine, is in agreement with the characteristics of the *Betaproteobacteria* (Busse & Auling, 1988).

In this study, *P. mephitica* CCUG 2513^T was studied for its exact taxonomic position along with *J. lividum* CCUG 2344^T. The 16S rRNA gene sequences (both 1388 bp) were studied as described by Kämpfer *et al.* (2003) and shared 99.8 % similarity.

For polar lipid, quinone and polyamine analyses, cells were grown on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2). Analyses were carried out as described previously (Busse & Auling, 1988; Tindall, 1990a, b; Altenburger *et al.*, 1996; Stolz *et al.*, 2007). The major phospholipids were detected to be phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. In addition, moderate amounts of an unknown aminolipid and an unknown phospholipid and minor to trace amounts of another unknown aminolipid and five unknown polar lipids were found (Supplementary Fig. S1, available in IJSEM Online). *J. lividum* CCUG 2344^T exhibited the same profile (results not shown), and both

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 2513^T is AM748811.

A two-dimensional TLC of the polar lipids of *P. mephitica* CCUG 2513^T is available as supplementary material with the online version of this paper.

profiles were in perfect agreement with those reported for the type strains of *J. lividum* and *Janthinobacterium agaricidamnosum* (Lincoln *et al.*, 1999), although these authors did not detect minor components. The quinone system of *P. mephitica* CCUG 2513^T consisted of 2 % ubiquinone Q-7 and 98 % Q-8 and the polyamine pattern also exhibited the characteristics of members of the *Betaproteobacteria* [$\mu\text{mol (g dry weight)}^{-1}$: 2-hydroxyputrescine, 19.4; putrescine, 71.7; cadaverine, 0.3; spermidine, 1.0; spermine, 0.6].

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI, Microbial ID; Kämpfer & Kroppenstedt, 1996). The fatty acid profiles of *P. mephitica* CCUG 2513^T and *J. lividum* CCUG 2344^T are shown in Table 1. No significant differences were found between the fatty acid profiles of the two strains.

All these chemotaxonomic traits clearly distinguish *P. mephitica* CCUG 2513^T from members of the genus *Pseudomonas sensu stricto*, which show a quinone system containing ubiquinone Q-9 (Yokota *et al.*, 1992) and a polyamine pattern with the predominant compounds putrescine and spermidine (Busse & Auling, 1988), and are in excellent agreement with the characteristics of *J. lividum*.

Physiological/biochemical tests were performed with methods described previously (Kämpfer *et al.*, 1991). Strains CCUG 2344^T and CCUG 2513^T shared the following biochemical characteristics. L-Alanine *p*-nitroanilide (pNA), bis-*p*-nitrophenyl (pNP) phosphate, bis-pNP phenylphosphonate, pNP β -D-glucopyranoside and L-proline pNA are hydrolysed on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not hydrolysed: pNP β -D-galactopyranoside, pNP β -D-glucuronide, pNP α -D-glucopyranoside, pNP β -D-xylopyranoside, bis-pNP phosphorylcholine and γ -L-glutamate pNA. The following compounds are used as sole sources of carbon: *N*-acetylgalactosamine, L-arabinose,

L-arbutin, D-fructose, D-galactose, D-glucose, maltose, D-ribose, sucrose, D-xylose (weakly), D-mannitol (weakly), D-sorbitol (weakly), citrate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, 2-oxoglutarate, pyruvate (weakly), L-alanine (weakly), L-proline (weakly) and L-serine (weakly). The following compounds are not utilized on the basis of the method described by Kämpfer *et al.* (1991): D-gluconate, acetate, propionate, *cis*- and *trans*-aconitate, 4-aminobutyrate, itaconate, mesaconate, β -alanine, L-aspartate, L-leucine, L-ornithine, L-serine, *N*-acetylglucosamine, D-cellobiose, D-mannose, α -D-melibiose, L-rhamnose, salicin, trehalose, adonitol, inositol, maltitol, putrescine, adipate, azelate, suberate, L-histidine, L-phenylalanine and L-tryptophan. Acids are produced from D-glucose, sucrose (weakly) and L-arabinose. No acids are produced from lactose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose.

On the basis of these results, it is clear that *P. mephitica* CCUG 2513^T is not a member of the genus *Pseudomonas sensu stricto* and, hence, we propose that the name *Pseudomonas mephitica* Claydon and Hammer 1939 is a later heterotypic synonym of *Janthinobacterium lividum* (Eisenberg 1891) De Ley *et al.* 1978 and that *P. mephitica* CCUG 2513^T should be assigned to *J. lividum* based on: (i) 99.8 % 16S rRNA gene sequence similarity between *J. lividum* CCUG 2344^T (=DSM 1522^T) and *P. mephitica* CCUG 2513^T (our confirmation: GenBank accession numbers Y08846 and AM748811, respectively), (ii) identical quinone systems, (iii) identical polar lipid profiles, (iv) highly similar polyamine patterns, (v) similar fatty acid profiles (Table 1) and (vi) identical biochemical test results.

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Table 1. Cellular fatty acid compositions (%) of *J. lividum* CCUG 2344^T and *P. mephitica* CCUG 2513^T

Fatty acid	<i>J. lividum</i> CCUG 2344 ^T	<i>P. mephitica</i> CCUG 2513 ^T
C _{10:0}	0.5	0.4
C _{10:0} 3-OH	2.9	4.6
C _{12:0}	4.3	3.6
C _{12:0} 2-OH	0.5	1.9
C _{14:0}	0.7	1.0
C _{16:1} ω 7c*	41.9	43.8
C _{16:0}	39.0	33.1
C _{17:0} cyclo	5.2	5.4
C _{18:1} ω 7c	4.3	4.0
iso-C _{19:0}	0.8	2.0

*Fatty acid C_{16:1} ω 7c is included in summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH).

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