Proposal of *Hymenobacter norwichensis* sp. nov.,
classification of '*Taxeobacter ocellatus*',
'*Taxeobacter gelupurpurascens*' and '*Taxeobacter*
chitinovorans' as *Hymenobacter ocellatus* sp. nov.,
Hymenobacter gelupurpurascens sp. nov. and
Hymenobacter chitinivorans sp. nov., respectively,
and emended description of the genus
Hymenobacter Hirsch *et al.* 1999

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Two airborne bacterial isolates, NS/2 and NS/50^T, were examined in order to determine their taxonomic position. Their almost complete 16S rRNA gene sequences shared 95.9% similarity. Sequence comparisons demonstrated that their next relatives are species of the genus *Hymenobacter* (93.6–95.7% similarity) and the strains '*Taxeobacter chitinovorans*' Txc1^T, '*Taxeobacter gelupurpurascens*' Tgx1^T and '*Taxeobacter ocellatus*' Myx 2105^T (90.5–96.4%). Phylogenetic calculations indicated that these five strains together with the three recognized *Hymenobacter* species form a separate line of descent within the family 'Flexibacteraceae'. Isolates NS/2 and NS/50^T, as well as '*Taxeobacter chitinovorans*' Txc1^T, '*Taxeobacter gelupurpurascens*' Tgx1^T and '*Taxeobacter ocellatus*' Myx 2105^T, possessed the characteristics of the genus *Hymenobacter*, the quinone system menaquinone MK-7 and a polyamine pattern with the major polyamine being sym-homospermidine. Each of the five strains had complex, unique polar lipid profiles, with phosphatidylethanolamine and several unknown aminophospho-, amino-, phospho-, glyco- and polar lipids of which several compounds were also found in established *Hymenobacter* species. All the strains studied possessed fatty acids characteristic of *Hymenobacter* species, including major acids iso-C_{15:0}, anteiso-C_{15:0}, C_{16:1ω5c}, summed feature 3 (C_{16:1ω7c}/iso-C_{15:0} 2-OH) and summed feature 4 (iso-C_{17:1} l/anteiso-C_{17:1} B). The five strains could be distinguished from each other and from the three established species of the genus *Hymenobacter* based on relatively low 16S rRNA gene sequence similarities (<97%), unique polar lipids and differing fatty acid profiles and physiological characteristics. In conclusion, the description of four novel species of the genus *Hymenobacter* appears to be justified, for which the names *Hymenobacter norwichensis* sp. nov. (type strain NS/50^T=LMG 21876^T=DSM 15439^T), *Hymenobacter chitinivorans* sp. nov. (type strain Txc1^T=LMG 21951^T=DSM 11115^T), *Hymenobacter gelupurpurascens* sp. nov. (type strain Tgx1^T=LMG 21873^T=DSM 11116^T) and *Hymenobacter ocellatus* sp. nov. (type strain Myx 2105^T=Txo1^T=LMG 21873^T=DSM 11117^T) are proposed. For strain NS/2, a description only is provided without proposal of a name because its status as a novel species was not demonstrated unambiguously.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NS/2 and NS/50^T are AJ549284 and AJ549285, respectively.

During a study on airborne bacteria in the Sainsbury Centre for Visual Arts in Norwich, UK, numerous bacterial isolates were collected (Brimblecombe *et al.*, 1999). Selected strains were

subjected to chemotaxonomic characterization and partial 16S rRNA gene sequence analysis. Based on these results, the isolates were identified as members of numerous different genera including *Frigoribacterium* (Kämpfer *et al.*, 2000), *Micrococcus* (Wieser *et al.*, 2002), *Sphingomonas* (Busse *et al.*, 2003), *Bacillus*, *Terrabacter*, *Nocardioides*, *Brevibacterium*, *Curtobacterium*, *Cellulomonas*, *Staphylococcus*, *Paucimonas*, *Duganella*, *Psychrobacter* and 'Taxeobacter'/Hymenobacter (O. Kim, U. Ulrych & H.-J. Busse, unpublished data). Two strains, NS/2 and NS/50^T, identified as members of the 'Taxeobacter'/Hymenobacter lineage, were subjected to a more detailed characterization.

Members of the 'Taxeobacter'/Hymenobacter lineage belong to the *Bacteroidetes* line of descent. Cultured representatives of this lineage are *Hymenobacter roseosalivarius*, *Hymenobacter actinosclerus*, *Hymenobacter aerophilus*, 'Taxeobacter chitinovorans', 'Taxeobacter gelupurpurascens' and 'Taxeobacter ocellatus' (Hirsch *et al.*, 1998; Collins *et al.*, 2000; Buczolits *et al.*, 2002; Reichenbach, 1992). As neither the genus name 'Taxeobacter' nor the species names 'Taxeobacter chitinovorans', 'Taxeobacter gelupurpurascens' and 'Taxeobacter ocellatus' have been validly published, these names do not have standing in nomenclature. In contrast to other taxa belonging to the phylum *Bacteroidetes* they were reported to have genomic DNA with a high G + C content (55–61 mol%) and to be able to survive under unfavourable conditions such as exposure to increased desiccation (Reichenbach, 1992; Hirsch *et al.*, 1998; Buczolits *et al.*, 2002) and high levels of radiation (Collins *et al.*, 2000). Also, the fact that, among 319 cloned 16S rRNA gene sequences from an air sampling campaign in Salt Lake City (UT, USA), thirteen sequences showed the highest similarities with representatives of this lineage (http://www-bio.llnl.gov/bbrp/html/SLC_air_clones3.html) is in agreement with their assumed desiccation resistance.

Classification of the isolates employed methods used previously for the description of *H. aerophilus* (Buczolits *et al.*, 2002). The strains were characterized biochemically according to Kämpfer *et al.* (1991), with the modification reported by Buczolits *et al.* (2002). Polyamines, quinones, polar lipids and fatty acids were extracted and analysed as described by Busse & Auling (1988), Busse *et al.* (1997), Tindall (1990), Altenburger *et al.* (1996), Ventosa *et al.* (1993) and Kämpfer *et al.* (1997). Morphological, physiological and biochemical traits are summarized in the species descriptions and Table 1.

PCR and sequencing of the 16S rRNA gene of airborne strains NS/2 and NS/50^T resulted in continuous stretches of 1436 and 1442 nucleotides, respectively. After sequence comparison using FASTA (Pearson & Lipman, 1988), the two strains shared 95.9% 16S rRNA gene sequence similarity and showed highest similarities (97–98%) to sequences of uncultured strains that were obtained from studies in spacecraft assembly facilities. Strain NS/2 exhibited the highest 16S rRNA gene sequence similarity to *Hymenobacter rigui* WPCB131^T (98.5%; manuscript under review at the

same time as our manuscript) and 'Taxeobacter gelupurpurascens' Txg1^T (96.4%), moderate similarities to *H. actinosclerus* CCUG 39621^T (95.7%), 'Taxeobacter chitinovorans' Txc1^T (94.8%), *H. aerophilus* DSM 13606^T (94.2%) and *H. roseosalivarius* DSM 11622^T (93.6%) and low similarity to 'Taxeobacter ocellatus' Myx 2105^T (90.6%). Strain NS/50^T exhibited moderate similarities to *H. actinosclerus* CCUG 39621^T (95.5%), 'Taxeobacter chitinovorans' Txc1^T (95.3%), *Hymenobacter rigui* WPCB131^T (95.3%), *H. roseosalivarius* DSM 11622^T (94.9%), 'Taxeobacter gelupurpurascens' Txg1^T (94.7%) and *H. aerophilus* DSM 13606^T (93.9%) and low similarity to 'Taxeobacter ocellatus' Myx 2105^T (90.5%). These data indicate that strain NS/50^T, and perhaps also NS/2, are representatives of so far unrecognized species of the genus *Hymenobacter*. In order to classify strains 'Taxeobacter chitinovorans' Txc1^T, 'Taxeobacter gelupurpurascens' Txg1^T and 'Taxeobacter ocellatus' Myx 2105^T in more detail, we included them in our study. 16S rRNA gene sequence similarities between the 'Taxeobacter' strains were in the range 90.9–95.0%, clearly demonstrating that each of them represents a single species. Based on 16S rRNA gene sequence similarities, the closest related species of the strains studied here, other than *Hymenobacter* spp., included *Adhaeribacter aquaticus* MBRG1.5^T (87.2–89.2%) and *Pontibacter actiniarum* KMM 6156^T (86.3–88.1%). Phylogenetic calculations were in good agreement with the results from sequence comparisons (Fig. 1).

The five strains exhibited similar polyamine patterns, with the predominant compound being *sym*-homospermidine [9.6–32.8 µmol (g dry wt)⁻¹] and minor amounts of spermidine [1.2–1.6 µmol (g dry wt)⁻¹]. Some strains also contained minor amounts of cadaverine and spermine. Their quinone systems consisted of menaquinone MK-7 (95–100%) and MK-6 (0–5%). The polyamine patterns and quinone systems are both in accordance with the characteristics reported for other species of the genus *Hymenobacter* (Buczolits *et al.*, 2002).

The polar lipid profiles of the five strains consisted of 8–13 components (Table 2). Each of the five strains displayed a distinct polar lipid profile, distinguishing them from *H. roseosalivarius* DSM 11622^T, *H. actinosclerus* CCUG 39621^T (Table 2), *H. aerophilus* DSM 13606^T and an unnamed *Hymenobacter* strain I/74-Cor2 (DSM 13611) (Buczolits *et al.*, 2002). Strain Myx 2105^T exhibited the most distinct profile. It lacked the unknown aminophospholipid APL4 that was present in all the other strains and in contrast to all of them it contained three unknown aminolipids (Fig. 2). Among the polar lipids only phosphatidylethanolamine could be identified based on its chromatographic and staining behaviour. In four of five strains, no polar lipids could be detected that showed the chromatographic and staining behaviour of diphosphatidylglycerol. It is important to emphasize this observation because strains Txc1^T and Txg1^T as well as *H. roseosalivarius* were reported to contain trace amounts of diphosphatidylglycerol (Hirsch *et al.*, 1998). However, we were also able to detect a spot showing

Table 1. Characteristics that distinguish *Hymenobacter* species and strains

Taxa: 1, NS/50^T; 2, NS/2; 3, Txc1^T; 4, Txc1^T; 5, Myx 2105^T; 6, *H. actinosclerus* CCUG 39621^T (data from Collins *et al.*, 2000; Buczolits *et al.*, 2002); 7, *H. roseosalivarius* (DSM 11622^T and AA 718; Hirsch *et al.*, 1998); 8, *H. aerophilus* (I/26-Cor1^T and I/32A-Cor1; Buczolits *et al.*, 2002); 9, I/74-Cor2 (Buczolits *et al.*, 2002). For miniaturized tests, media were inoculated with a 3-fold-diluted cell suspension (Buczolits *et al.*, 2002) and incubated at 15 °C for 28 days. Other tests were incubated at room temperature and 28 °C. +, Positive; (+), weakly positive; –, negative; V, variable; NG, no growth; ND, not determined. None of the strains assimilated L-arabinose, adonitol, i-inositol, D-sorbitol, putrescine, azelate, 4-aminobutyrate, β -alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate or hydrolysed *p*-nitrophenyl (pNP) phosphoryl-choline or pNP β -D-galactopyranoside. None of the strains haemolysed sheep blood at 28 or 37 °C.

Characteristic	1	2	3	4	5	6	7	8	9
Growth at/in:									
1 % NaCl	–	–	(+)	(+)	+	ND	+*	+	(+)
3 % NaCl	–	–	(+)	(+)	+	ND	ND	(+)	–
Starch	+	+	(+)	(+)	+	+	ND	+	+
Casein	(+)	NG	+	(+)	+	ND	ND	V	–
Tyrosine	+	NG	+	NG	+	ND	ND	+	+
4 °C	(+)	–	+	(+)	–	–†	+*	+	+
37 °C	–	–	–	–	+	+‡	–	–	–
Assimilation of:									
<i>N</i> -Acetyl-D-glucosamine, gluconate	–	–	–	–	–	(+)	–	–	–
<i>p</i> -Arbutin, D-cellobiose, D-ribose	(+)	–	–	–	–	(+)	–	–	–
D-Fructose	(+)	–	–	–	–	–	–	(+)	–
D-Galactose, D-xylose	(+)	–	–	–	–	–	–	–	–
D-Glucose, D-mannose	(+)	–	–	–	–	(+)	–	(+)	(+)
L-Rhamnose, maltitol	–	–	–	–	–	–	–	–	(+)
Sucrose	–	–	–	–	–	–	–	(+)	(+)
Salicin, D-maltose, D-trehalose, D-mannitol	(+)	–	–	–	–	–	–	–	(+)
Acetate	–	–	–	–	–	(+)	(+)	(+)	(+)
Propionate	–	(+)	–	–	–	(+)	(+)	(+)	(+)
Glutarate	–	(+)	–	–	–	(+)	(+)	–	–
Pyruvate	–	–	–	–	–	(+)	(+)	–	–
<i>cis</i> -Aconitate, adipate, citrate, fumarate, DL-3-hydroxybutyrate, L-malate, itaconate, DL-lactate, mesaconate, oxoglutarate, suberate, L-alanine, L-proline, L-phenylalanine	–	–	–	–	–	(+)	–	–	–
Hydrolysis of:									
pNP β -D-glucuronide	–	–	–	–	+	–	–	–	–
pNP phosphate, pNP α -D-glucopyranoside, L-proline <i>p</i> -nitroanilide (pNA)	(+)	(+)	–	+	+	(+)	(+)	(+)	(+)
pNP β -D-glucopyranoside	(+)	–	–	–	+	–	–	–	(+)
bis-pNP phosphate	(+)	–	–	–	+	(+)	(+)	(+)	(+)
pNP phenyl-phosphonate	(+)	–	–	–	–	(+)	–	(+)	(+)
L-Alanine pNA	(+)	(+)	+	+	+	(+)	(+)	(+)	(+)
L-Glutamate- γ -3-carboxy pNA	–	–	–	–	–	(+)	–	(+)	(+)

*Hirsch *et al.* (1998).

†Collins *et al.* (2000) reported no growth at 5 °C.

‡Collins *et al.* (2000) reported growth at 42 °C.

the chromatographic and staining behaviour of diphosphatidylglycerol in the chromatographic analysis of *H. roseosalivarius* DSM 11622^T. This observation might be explained by different growth conditions. Due to poor growth on PYES medium, in contrast to the other strains analysed for polar

lipids, *H. roseosalivarius* DSM 11622^T was grown on R2A agar and the biomass was harvested by being scraped from the surface of the plate. In conclusion, it is recommended that, for comparison of polar lipid profiles of hymenobacters, standardized growth conditions should be used.

Fig. 1. Distance matrix phylogenetic tree of species of the genus *Hymenobacter* based on 16S rRNA gene sequences after multiple alignment using CLUSTAL_X (Thompson *et al.*, 1997). Sequences were manually edited to remove ambiguous nucleotides and gaps using BioEdit (Hall, 1999). Phylogenetic analyses were performed using DNAML in the PHYLIP package (Felsenstein, 1993). The tree was visualized using TreeView (Page, 1996). The sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup. Numbers at nodes are bootstrap values (100 replications). Only values greater than 65% confidence are shown. Bar, 0.01 substitutions per nucleotide position.

For fatty acid analyses, all strains were grown on trypticase soy agar but, because of poor growth on this medium, strains NS/2 and NS/50^T were grown on PYES agar (0.3% peptone from casein, 0.3% yeast extract, 0.23% disodium succinate; pH 7.2 at 25 °C). All strains contained a complex fatty acid profile consisting of predominantly branched and minor amounts of straight-chain acids (Table 3), as already reported for other *Hymenobacter* strains (Hirsch *et al.*, 1998; Buczolits *et al.*, 2002). Relative amounts of major fatty acids varied significantly among the five strains allowing their differentiation from each other and from the other *Hymenobacter* species. Double unsaturated fatty acids or high proportions of C_{17:1ω6c} as reported by Hirsch *et al.* (1998) for strains Txc1^T and Txg1^T could not be detected. This deviation might be related to different cultivation conditions but this can only be assumed because Hirsch *et al.* (1998) did not indicate the conditions under which biomass of Txc1^T and Txg1^T was grown for fatty acid extraction.

Based on the results presented here, it is obvious that strains NS/2, NS/50^T, Txc1^T, Txg1^T and Myx 2105^T each represents a single species. Strain Myx 2105^T might be considered as a representative of a separate genus because the 16S rRNA gene sequence similarities to the other four strains and the three established *Hymenobacter* species (90.5–92.1%) are rather low. Also, the polar lipid profile clearly distinguishes this strain from all other *Hymenobacter* species. However, the usefulness of the polar lipid profile for the proposal of a novel genus for Myx 2105^T should be substantiated by investigation of closely related strains that, so far, are not

available. Hence, it appears to be justified to classify strain Myx 2105^T as representing a novel species of the genus *Hymenobacter*, *Hymenobacter ocellatus* sp. nov. In addition, we also propose that strains NS/50^T, Txc1^T and Txg1^T represent novel species of the genus *Hymenobacter*, for which we propose the names *Hymenobacter norwichensis* sp. nov., *Hymenobacter chitinivorans* sp. nov. and *Hymenobacter gelipurpurascens* sp. nov., respectively. There was some discussion among the authors of this study about how to deal with strain NS/2. In another paper, *Hymenobacter rigui* WPCB131^T (Baik *et al.* 2006; this issue of IJSEM) is described with which strain NS/2 shares 98.5% 16S rRNA gene sequence similarity, indicating that they might be members of a single species. The question whether strains NS/2 and *Hymenobacter rigui* WPCB131^T are members of a single species can only be answered by results from DNA–DNA hybridization. As the paper describing *Hymenobacter rigui* WPCB131^T was reviewed at the same time, one option was to wait until publication of *Hymenobacter rigui* WPCB131^T and its accessibility for DNA–DNA hybridization, which would have caused significant delay in the publication of our data. As our studies presented here concentrate on the description of four novel species and the traits of NS/2 are complementary from our point of view, the clarification of the taxonomic status of NS/2 did not justify the delay in publication of the four novel *Hymenobacter* species. The second option was to remove all data concerning NS/2 from the manuscript, do the necessary DNA–DNA hybridizations and, if the results demonstrated that NS/2 is a representative of a distinct

Table 2. Polar lipid profiles of *H. actinosclerus* CCUG 39621^T, *H. roseosalivarius* DSM 11622^T, '*Taxeobacter*' strains Txc1^T, Tgx1^T, Myx 2105^T (=Txo1^T) and isolates NS/50^T and NS/2

Taxa: 1, Txc1^T; 2, Tgx1^T; 3, Myx 2105^T; 4, NS/2; 5, NS/50^T; 6, *H. actinosclerus* CCUG 39621^T; 7, *H. roseosalivarius* DSM 11622^T. +, Present; tr, present in trace amounts; ND, not detected. Abbreviations: APL1–6, unknown aminophospholipids; AL1–4, unknown aminolipids; PL, unknown phospholipid; DPG, diphosphatidylglycerol; GL1 and 2; unknown glycolipids; L2, L3, L5, L8, unknown polar lipids. For comparability, the same designations for unknown lipids were used as employed previously (Buczolits *et al.*, 2002) and for new unknown lipids we continued with the letter/number code. APL3 corresponds to APL reported to be present in *H. aerophilus*. All strains contained phosphatidylethanolamine, the unknown aminophospholipid APL3 and the unknown polar lipids L3 and L5.

Polar lipid	1	2	3	4	5	6	7
APL1	+	+	+	+	+	+	ND
APL2	+	+	+	+	+	+	ND
APL4	+	+	ND	+	+	+	+
APL5	ND	ND	ND	ND	ND	+	+
APL6	ND	ND	ND	ND	ND	+	ND
AL1	ND	ND	+	ND	ND	ND	ND
AL2	ND	ND	+	ND	ND	ND	ND
AL3	ND	ND	+	ND	ND	ND	ND
AL4	ND	ND	ND	+	ND	ND	ND
PL	+	ND	+	ND	ND	tr	+
DPG	ND	ND	ND	ND	ND	ND	+
GL1	+	+	+	+	+	+	ND
GL2	+	+	+	+	+	ND	ND
L2	+	+	+	ND	ND	+	+
L8	ND	+	+	+	+	+	ND

species, to describe it as a novel species later. However, if the degree of DNA–DNA relatedness were to demonstrate that strain NS/2 and *Hymenobacter rigui* WPCB131^T are members of a single species all characteristics of NS/2 would be lost for the scientific community because they may not justify an emended description of the novel species of which *Hymenobacter rigui* WPCB131^T is the type strain. Hence, we decided that the third option, to present all data on NS/2 in this study without proposing a name, was the best solution.

Traits of strain NS/2

Size of cells is approximately 3–4 × 0.8 µm. Cells are Gram-negative according to Gram-staining, KOH (3%) and aminopeptidase test. Colonies are brick-red-pigmented. Growth occurs on PYES and R2A agar but not on Czapek-Dox agar or MacConkey agar. Oxidase- and catalase-positive. Nitrate reduction is positive without production of gas. Negative for production of indole from tryptophan, arginine dihydrolase and urease. Positive in the API ZYM system for alkaline phosphatase, esterase C4, esterase lipase

Fig. 2. Polar lipid profile of strain Myx 2105^T. For abbreviations see Table 2.

C8, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -galactosidase; negative for lipase C14, trypsin, chymotrypsin, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Other characteristics are listed in Table 1. Sensitive to bacitracin (10 IU), chloramphenicol (30 µg), colistin sulphate (10 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 IU), vancomycin (30 µg), polymyxin B sulphate (300 IU) and tetracycline (10 µg). *sym*-Homospermidine is the predominant compound in the polyamine pattern. Quinone system is menaquinone MK-7. The polar lipid profile is composed of the major compound phosphatidylethanolamine and four unknown aminophospholipids, two glycolipids, an aminolipid and three polar lipids. The fatty acid profile predominantly contains methyl-branched acids of iso- and anteiso-type. Major fatty acids are (>5% in decreasing order) iso-C_{15:0}, summed feature 3 (C_{16:1} ω 7*c*/iso-C_{15:0} 2-OH), summed feature 4 (iso-C_{17:1} I/ anteiso-C_{17:1} B), anteiso-C_{15:0} and C_{16:1} ω 5*c*. Isolated from the air in the Sainsbury Centre for Visual Arts in Norwich (UK). Strain NS/2 has been deposited in two culture collections under the numbers DSM 15438 and LMG 21875.

The original description of the genus *Hymenobacter* (Hirsch *et al.*, 1998) was based on the characteristics of a single species and some unnamed strains. The genus currently contains three species (Hirsch *et al.*, 1998; Collins *et al.*, 2000; Buczolits *et al.*, 2002) and in this study we propose four more species. Hence, we think that an emended description of the genus *Hymenobacter* is needed.

Table 3. Fatty acid profiles of strains Txc1^T, Tgx1^T, Myx 2105^T, NS/50^T and NS/2^T

Strains: 1, Txc1^T; 2, Tgx1^T; 3, NS/2; 4, Myx 2105^T; 5, NS/50^T. Values are percentages of total fatty acids. Strains NS/50^T and NS/2 were grown on a medium containing disodium succinate as carbon source at 25 °C. For unsaturated fatty acids, the position of the double bond can be located by counting from the methyl (ω) end of the carbon chain. *Cis* is indicated by the suffix *c*. Summed features represent groups of two or three fatty acids that could not be separated by gas-liquid chromatography with the MIDI system. Summed features: 1, iso-C_{15:1} I/C_{13:0} 3-OH; 3, C_{16:1} ω 7c/iso-C_{15:0} 2-OH; 4, iso-C_{17:1} I/anteiso-C_{17:1} B.

Fatty acid	1	2	3	4	5
iso-C _{13:0}	0.6	—	—	0.6	—
C _{14:0}	—	—	0.3	—	1.3
Unknown 13.565	0.5	0.8	0.4	—	—
iso-C _{14:0}	0.5	—	0.6	1.5	0.8
iso-C _{15:1} G	0.6	0.8	—	—	—
anteiso-C _{15:1} A	—	1.1	1.0	—	—
Summed feature 1	2.1	—	2.8	2.7	0.8
iso-C _{15:0}	31.1	17.3	18.7	36.7	27.3
anteiso-C _{15:0}	3.7	23.1	16.2	3.9	10.6
iso-C _{15:0} 3-OH	4.1	2.3	2.0	4.3	2.2
C _{15:0} 2-OH	—	0.9	1.0	—	—
C _{15:1} ω 6c	—	—	—	—	0.5
C _{15:0}	—	—	—	—	0.5
iso-C _{16:1} H	2.0	3.7	2.0	1.3	1.4
iso-C _{16:0} 3-OH	—	—	0.3	—	—
C _{16:1} ω 5c	8.9	11.3	7.4	2.0	13.6
Summed feature 3	13.9	17.6	18.5	5.4	23.6
iso-C _{16:0}	1.4	2.7	1.3	2.9	1.2
C _{16:0}	—	—	1.0	0.6	2.2
C _{16:0} 3-OH	0.5	—	0.4	—	0.6
Unknown 16.580	—	—	0.5	—	—
iso-C _{17:0}	2.6	1.7	2.0	2.8	1.6
anteiso-C _{17:0}	—	2.0	1.3	—	—
iso-C _{17:0} 3-OH	6.2	3.7	3.6	6.8	3.0
C _{17:0} 2-OH	—	1.7	1.0	—	—
C _{17:1} ω 6c	—	—	0.5	1.2	—
iso-C _{17:1} ω 9c	0.7	—	0.5	0.6	—
Summed feature 4	20.8	9.4	17.2	26.9	8.3

Emended description of the genus *Hymenobacter* Hirsch *et al.* 1999

In addition to the characteristics summarized by Hirsch *et al.* (1998), several other genus-specific characteristics can be identified. Temperature maximum is up to 42 °C but most strains do not grow at this temperature. All members have a polar lipid profile containing phosphatidylethanolamine, an unknown aminophospholipid (APL3) and two unknown polar lipids (L3, L5) and a mixture of several other unknown aminophospholipids, aminolipids, phospholipids, glycolipids and polar lipids. The fatty acid profile consists of predominantly branched fatty acids of the

iso- and anteiso-type with iso-C_{15:0}, summed feature 3 (C_{16:1} ω 7c/iso-C_{15:0} 2-OH) and summed feature 4 (iso-C_{17:1} I/anteiso-C_{17:1} B) always present in moderate to major amounts. *sym*-Homospermidine is the major compound in the polyamine pattern and the quinone system is MK-7. Highly sensitive to the action of numerous antibiotics. The DNA G + C content of species within the genus ranges from 55 to 65 mol%. Currently comprises seven species, *Hymenobacter actinosclerus*, *Hymenobacter aerophilus*, *Hymenobacter norwichensis*, *Hymenobacter chitinivorans*, *Hymenobacter gelipurpurascens*, *Hymenobacter ocellatus* and *Hymenobacter roseosalivarius* (type species of the genus).

Description of *Hymenobacter ocellatus* sp. nov.

Hymenobacter ocellatus (o.cel.la'tus. L. masc. adj. *ocellatus* showing little eyes, referring to the bright granules at the cell poles).

The description is based on characteristics reported by Reichenbach (1992) and our own data. Size of cells is approximately 3–6 × 1 µm. Cells are Gram-negative according to Gram-staining, KOH (3 %) and aminopeptidase test. Colonies are brick-red-pigmented. Growth occurs on PYES agar, Czapek-Dox agar and R2A agar but not on MacConkey agar. Oxidase- and catalase-positive. Nitrate reduction is weakly positive without production of N₂. Negative for production of indole from tryptophan, arginine dihydrolase and urease. Positive in the API ZYM system for alkaline phosphatase, esterase lipase C8 (weakly), leucine arylamidase (weakly) and naphthol-AS-BI-phosphohydrolase (weakly); negative for esterase C4, lipase C14, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Other characteristics are listed in Table 1. Sensitive to bacitracin (10 IU), chloramphenicol (30 µg), colistin sulphate (10 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 IU), vancomycin (30 µg), polymyxin B sulphate (300 IU) and tetracycline (10 µg). *sym*-Homospermidine is the predominant compound in the polyamine pattern. Quinone system is menaquinone MK-7. The polar lipid profile is composed of the major compound phosphatidylethanolamine and three unknown aminophospholipids, three aminolipids, two glycolipids, a phospholipid and four polar lipids. The fatty acid profile predominantly contains methyl-branched acids of iso- and anteiso-type. Major fatty acids are (> 5 % in decreasing order) iso-C_{15:0}, summed feature 4 (iso-C_{17:1} I/anteiso-C_{17:1} B), iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1} ω 7c/iso-C_{15:0} 2-OH). The G + C content of the DNA is approximately 65 mol%.

The type strain, Myx 2105^T (=Txc1^T=LMG 21873^T=DSM 11117^T), was isolated from South African soil.

Description of *Hymenobacter gelipurpurascens* sp. nov.

Hymenobacter gelipurpurascens (ge.li.pur.pu.ras'cens. L. n. gelus -us the ice cold; L. part. adj. purpurascens becoming of a purple colour; N.L. part. adj. gelipurpurascens becoming purple in the cold).

The description is based on characteristics reported by Reichenbach (1992) and our own data. Size of cells is approximately $2 \times 0.5 \mu\text{m}$. Cells are Gram-negative according to Gram-staining, KOH (3 %) and aminopeptidase test. Colonies are brick-red-pigmented but at temperatures between 2 and 6 °C during growth on a certain medium colonies are deep blood-red (Reichenbach, 1992). Growth occurs on PYES agar, Czapek-Dox agar and R2A agar but not on MacConkey agar. Oxidase- and catalase-positive. Nitrate reduction is weakly positive without production of N_2 . Negative for production of indole from tryptophan, arginine dihydrolase and urease. Positive in the API ZYM system for alkaline phosphatase, esterase C4 (weakly), esterase lipase C8 (weakly), leucine arylamidase, valine arylamidase (weakly) and naphthol-AS-BI-phosphohydrolase (weakly); negative for lipase C14, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Other characteristics are listed in Table 1. Sensitive to bacitracin (10 IU), chloramphenicol (30 μg), colistin sulphate (10 μg), erythromycin (15 μg), fusidic acid (10 μg), gentamicin (10 μg), kanamycin (30 μg), penicillin G (10 IU), vancomycin (30 μg), polymyxin B sulphate (300 IU) and tetracycline (10 μg). *sym*-Homospermidine is the predominant compound in the polyamine pattern. Quinone system is menaquinone MK-7. The polar lipid profile is composed of the major compound phosphatidylethanolamine and four unknown aminophospholipids, two glycolipids, a phospholipid and three polar lipids. The fatty acid profile predominantly contains methyl-branched acids of iso- and anteiso-type. Major fatty acids are (>5 % in decreasing order) anteiso- $\text{C}_{15:0}$, summed feature 3 ($\text{C}_{16:1}\omega 7\text{c}/\text{iso-}\text{C}_{15:0}$ 2-OH), iso- $\text{C}_{15:0}$, $\text{C}_{16:1}\omega 5\text{c}$ and summed feature 4 (iso- $\text{C}_{17:1}$ I/anteiso- $\text{C}_{17:1}$ B). The G + C content of the DNA is approximately 57–58 mol%.

The type strain, Txl^T (= LMG 21873^T = DSM 11116^T), was isolated from soil.

Description of *Hymenobacter chitinivorans* sp. nov.

Hymenobacter chitinivorans (chi.ti.ni.vo'rans. N.L. neut. n. chitinum chitin; L. part. adj. vorans devour; N.L. part. adj. chitinivorans devouring chitin).

The description is based on characteristics reported by Reichenbach (1992) and our own data. Size of cells is approximately $4 \times 0.8 \mu\text{m}$. Cells are Gram-negative according to Gram-staining, KOH (3 %) and aminopeptidase test. Colonies are brick-red-pigmented. Growth occurs on PYES

agar, Czapek-Dox agar and R2A agar but not on MacConkey agar. Oxidase- and catalase-positive. Nitrate reduction is weakly positive without production of N_2 . Negative for production of indole from tryptophan, arginine dihydrolase and urease. Chitin is degraded. Positive in the API ZYM system for alkaline phosphatase, esterase C4 (weakly), esterase lipase C8 (weakly), leucine arylamidase and naphthol-AS-BI-phosphohydrolase (weakly); negative for lipase C14, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Other characteristics are listed in Table 1. Sensitive to bacitracin (10 IU), chloramphenicol (30 μg), colistin sulphate (10 μg), erythromycin (15 μg), fusidic acid (10 μg), gentamicin (10 μg), kanamycin (30 μg), penicillin G (10 IU), vancomycin (30 μg), polymyxin B sulphate (300 IU) and tetracycline (10 μg). *sym*-Homospermidine is the predominant compound in the polyamine pattern. Quinone system is menaquinone MK-7. The polar lipid profile is composed of the major compound phosphatidylethanolamine and four unknown aminophospholipids, two glycolipids, a phospholipid and three polar lipids. The fatty acid profile predominantly contains methyl-branched acids of iso- and anteiso-type. Major fatty acids are (>5 % in decreasing order), iso- $\text{C}_{15:0}$, summed feature 4 (iso- $\text{C}_{17:1}$ I/anteiso- $\text{C}_{17:1}$ B), summed feature 3 ($\text{C}_{16:1}\omega 7\text{c}/\text{iso-}\text{C}_{15:0}$ 2-OH), $\text{C}_{16:1}\omega 5\text{c}$ and iso- $\text{C}_{17:0}$ 3-OH. The G + C content of the DNA is approximately 61 mol%.

The type strain, Txc1^T (= LMG 21951^T = DSM 11115^T), was isolated from soil.

Description of *Hymenobacter norwichensis* sp. nov.

Hymenobacter norwichensis (nor.wi.chen'sis. N.L. masc. adj. norwichensis pertaining to Norwich, a city in England, where the type strain was isolated from the air in the Sainsbury Centre for Visual Arts).

Size of cells is approximately $2\text{--}3 \times 0.8 \mu\text{m}$. Cells are Gram-negative according to Gram-staining, KOH (3 %) and aminopeptidase test. Colonies are brick-red-pigmented. Growth occurs on PYES agar and R2A agar but not on Czapek-Dox agar or MacConkey agar. Oxidase- and catalase-positive. Nitrate reduction is positive without production of N_2 . Negative for production of indole from tryptophan, arginine dihydrolase and urease. Positive in the API ZYM system for alkaline phosphatase, esterase C4 (weakly), esterase lipase C8 (weakly), leucine arylamidase, valine arylamidase (weakly), acid phosphatase (weakly), naphthol-AS-BI-phosphohydrolase and β -glucosidase (weakly); negative for lipase C14, cystine arylamidase, trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Other characteristics are listed in Table 1. Sensitive to bacitracin (10 IU), chloramphenicol (30 μg), colistin sulphate (10 μg), erythromycin

(15 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 IU), vancomycin (30 µg), polymyxin B sulphate (300 IU) and tetracycline (10 µg). *sym*-Homospermidine is the predominant compound in the polyamine pattern. Quinone system is menaquinone MK-7. The polar lipid profile is composed of the major compound phosphatidylethanolamine and four unknown aminophospholipids, two glycolipids and three polar lipids. The fatty acid profile predominantly contains methyl-branched acids of iso- and anteiso-type. Major fatty acids are (>5% in decreasing order) iso-C_{15:0}, summed feature 3 (C_{16:1}ω7*c*/iso-C_{15:0} 2-OH), C_{16:1}ω5*c*, anteiso-C_{15:0} and summed feature 4 (iso-C_{17:1} I/anteiso-C_{17:1} B).

The type strain, NS/50^T (=LMG 21876^T=DSM 15439^T), was isolated from the air in the Sainsbury Centre for Visual Arts in Norwich (UK).

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