

Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov.

Sandra Buczolits,^{1,2} Ewald B. M. Denner,² Dietmar Vybiral,²
Monika Wieser,^{1,2} Peter Kämpfer³ and Hans-Jürgen Busse^{1,2}

¹ Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, Veterinärplatz 1, A-1210 Wien, Austria

² Institut für Mikrobiologie und Genetik, Universität Wien, A-1030 Wien, Austria

³ Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35390 Giessen, Germany

Author for correspondence: Hans-Jürgen Busse. Tel: +43 1 25077 2128. Fax: +43 1 25077 2190. e-mail: Hans-Juergen.Busse@vu-wien.ac.at

Three aerobic, Gram-negative, rod-shaped, non-spore-forming, red-pigmented, airborne bacteria (I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2) collected in the Museo Correr (Venice, Italy) were investigated to determine their taxonomic status by analysing their biochemical, physiological and chemotaxonomic features and the G+C content of genomic DNA and by comparing their genomic fingerprints. Additionally, the almost complete 16S rRNA gene sequence of strain I/26-Cor1^T was analysed. The three strains were nearly identical in their morphological, physiological, biochemical and chemotaxonomic properties. The strains contained a menaquinone system with the predominant menaquinone MK-7 and a fatty acid profile with C15:0 anteiso, C15:0 iso and C16:1 predominant. Phosphatidylethanolamine and several unidentified lipids were detected in the polar lipid profiles. The polyamine pattern consisted of *sym*-homospermidine as the major compound. *meso*-Diaminopimelic acid was found as the characteristic cell-wall diamino acid. The DNA base composition of the three strains ranged from 60 to 63 mol% G+C. Phylogenetically, strain I/26-Cor1^T was most closely related to *Hymenobacter actinosclerus* (95.8% 16S rRNA gene sequence similarity). Physiological and genomic characteristics indicated that the two strains I/26-Cor1^T and I/32A-Cor1 are representatives of the same species. The phylogenetic distance to any validly described taxon as indicated by 16S rRNA gene sequence similarities demonstrates that I/26-Cor1^T and I/32A-Cor1 represent a novel species, for which the name *Hymenobacter aerophilus* sp. nov. is proposed, with the type strain I/26-Cor1^T (= DSM 13606^T = LMG 19657^T). I/32A-Cor1 (= DSM 13607 = LMG 19658) is another strain of the species *Hymenobacter aerophilus*. Since the taxonomic status of strain I/74-Cor2 within the genus *Hymenobacter* was not determined unambiguously, it is designated *Hymenobacter* sp. I/74-Cor2 (= DSM 13611 = LMG 19659).

Keywords: *Hymenobacter aerophilus* sp. nov., phylogeny, chemotaxonomy, physiological/biochemical traits

INTRODUCTION

The recognition of the *Cytophaga-Flavobacterium-Bacteroides* group as a separate line of descent within the domain *Bacteria* has been clearly demonstrated by different rRNA-based studies (Bauwens & De Ley,

1981; Paster *et al.*, 1985; Segers *et al.*, 1993b; Weisburg *et al.*, 1985; Nakagawa & Yamasato, 1993; Woese *et al.*, 1990; Bernardet *et al.*, 1996). It contains numerous taxa including the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, *Sphingobacterium* (Yano *et al.*, 1982), *Weeksella* (Holmes *et al.*, 1986), *Sphingobacterium* (Takeuchi & Yokota, 1992), *Haliscomenobacter*, *Microscilla* (Reichenbach, 1992), *Capnocytophaga*, *Riemerella* (Segers *et al.*, 1993a), *Bergeyella*, *Chryseobacterium*, *Empedobacter* (Vandamme *et al.*, 1994b), *Ornithobacterium* (Vandamme *et al.*, 1994a),

Abbreviation: CasMM medium, casein minimal mineral medium.

The EMBL accession number for the 16S rRNA gene sequence of strain I/26-Cor1^T is AJ276901.

Bacteroides (Paster *et al.*, 1994), *Psychroserpens*, *Gelidibacter* (Bowman *et al.*, 1997), *Polaribacter* (Gosink *et al.*, 1998) and *Psychroflexus* (Bowman *et al.*, 1998). Common to all these genera is the relatively low G+C content of their genomic DNA (27–47 mol%). Recently, the genus *Hymenobacter* and species *Hymenobacter roseosalivarius* were described (Hirsch *et al.*, 1998). Together with *Hymenobacter actinosclerulus* (Collins *et al.*, 2000) and the non-validly described strains of 'Taxeobacter' (Reichenbach, 1992), *Hymenobacter roseosalivarius* represents a deep branch within the *Cytophaga-Flavobacterium-Bacteroides* group (Hirsch *et al.*, 1998). The separate position of these strains is displayed by the high G+C content of their genomic DNA (55–65 mol%), which is unique for the *Cytophaga-Flavobacterium-Bacteroides* group.

In the course of a study on the indoor air in different museums, which was supported by the European Commission in the R&D Programme Environment and Climate, three Gram-negative, red-pigmented, airborne bacterial strains were collected in the Museo Correr in Venice, in February 1996 (strains I/26-Cor1^T and I/32A-Cor1) and in August 1996 (strain I/74-Cor2) (Camuffo *et al.*, 1999). Since these strains, from among a huge number of isolates, displayed unusual red pigmentation, growth behaviour and fatty acid profiles, they were selected for further characterization. Based on the results of genomic, physiological/biochemical and chemotaxonomic characterization, we have shown that the three strains are members of the genus *Hymenobacter* and we propose the species *Hymenobacter aerophilus* sp. nov. for strains I/26-Cor1^T and I/32A-Cor1. The third strain is described as *Hymenobacter* sp. I/74-Cor2.

METHODS

Sample collection, bacterial strains and cultural conditions. Airborne bacteria were collected in the Museo Correr in Venice (Italy) using a Biotest Hycon air-sampler RCS (Reuter-Centrifugal-Sampler) Plus. Bacteria were collected and cultivated on casein minimal mineral (CasMM) medium (Altenburger *et al.*, 1996) supplemented with cycloheximide (250 mg l⁻¹). Sampling strips were incubated at room temperature for 4–5 d. The bacterial isolates I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 were subcultured on PYES agar plates [3 g peptone from casein l⁻¹, 3 g yeast extract l⁻¹, 2.3 g disodium succinate l⁻¹, 15 g agar l⁻¹ (all from Merck), pH 7.2]. Biomass subjected to further analyses was grown in PYES medium at 28 °C for 3–4 d or on PYES agar at room temperature.

Morphological, physiological and biochemical characterization. Cell morphology and motility were examined by phase-contrast microscopy (Leitz Diaplan) from cultures of different growth stages. Gram behaviour was tested by the KOH-lysis test (Moaledji, 1986), the L-alanine-aminopeptidase test (Merck) and by staining as described by Smibert & Krieg (1994). Cell sizes were estimated using a Leitz DM RB microscope by DAPI staining according to

Porter & Feig (1990) as modified by Sherr *et al.* (1992) from cells grown on tryptone soy agar (TSA) (Oxoid) at 28 °C for 72 h. Conventional biochemical tests were performed as described by Smibert & Krieg (1994), including tests for oxidase, catalase, nitrate reduction, H₂S production, citrate utilization, indole production and urease activity. Additionally, oxidase activity was tested using Bactident-Oxidase test strips (Merck) according to the manufacturer's instructions. DNase production was examined from 72 h-cultures grown on DNase test agar (Oxoid) at 28 °C. Growth on various standard bacteriological media was tested by using MacConkey agar, Czapek–Dox agar and R2A agar (Oxoid) according to the manufacturer's instructions. Hydrolysis of Tween 80 was determined on PYES agar supplemented with 5% Tween 80 and haemolysis was examined on sheep-blood agar [40 g blood agar base l⁻¹ (Oxoid), pH 7.2, supplemented with 5% sheep blood]. Growth at different temperatures (4, 28 and 37 °C) was investigated on PYES agar plates. Tolerance towards NaCl was studied on PYES agar plates supplemented with 0.5, 1.0, 2.0, 4.0 and 10.0% (w/v) NaCl, pH 7.0, and incubated at room temperature. Casein hydrolysis was determined on casein agar (50 g skimmed milk powder l⁻¹, 15 g agar l⁻¹). Carbon-source utilization tests and qualitative enzyme tests were done on microtitre plates as described previously (Kämpfer *et al.*, 1991) but media were inoculated with 150 µl instead of 50 µl suspension containing the equivalent cell density. Incubation was done at 15 °C for 28 d. Enzymic characterization was performed using API ZYM galleries (bioMérieux) according to the manufacturer's instructions.

Susceptibility to antimicrobials. Antimicrobial susceptibility testing was performed by the agar-diffusion method using antibiotic-impregnated disks (Oxoid). Briefly, 100 µl of a bacterial suspension, with an optical density equivalent to that of a 0.5 McFarland standard, was plated onto PYE agar. The following antibiotics were tested (µg of antibiotic per disk in parentheses): chloramphenicol (30), colistin sulfate (10), erythromycin (15), fusidic acid (10), gentamicin (10), kanamycin (30), nitrofurantoin (100), penicillin G (10 IU), bacitracin (10), polymyxin B (300 IU), tetracycline (10) and vancomycin (10). Any sign of growth inhibition was scored as sensitivity to that antibiotic. Resistance to an antimicrobial drug was indicated if no inhibition zone was observed.

Chemotaxonomic investigations. Menaquinones were extracted and analysed as described previously (Tindall, 1990). Polar lipids were extracted and analysed by two-dimensional TLC according to Ventosa *et al.* (1993). Cellular fatty acid methyl esters were analysed according to Kämpfer *et al.* (1997). Detection of the diagnostic cell-wall diamino acid was performed by the method of Schleifer (1985). Extraction and detection of polyamine patterns were performed as described by Busse & Auling (1988) and Busse *et al.* (1997). For pigment analysis, 100 mg cells grown on TSA for 72 h at 28 °C were scraped from the agar surface and placed into a small (5 ml), Teflon-sealed glass vial. Acetone (2 ml) was added to extract acetone-soluble pigments. After centrifugation at 10000 g at 4 °C for 5 min, the absorbance of the supernatant was scanned using a spectrophotometer (Hitachi S-2000); pure acetone was used as blank.

Determination of the G+C content of DNA. Isolation of genomic DNA was done as described by Auling *et al.* (1986). Purified DNA was dissolved in Nuclease P1 buffer (40 mM

sodium acetate, 2 mM ZnSO₄, pH 5.3). Determination of the G+C content was done by HPLC as described previously (Kaneko *et al.*, 1986).

Extraction of DNA and PCR amplification. Two loops of biomass were scraped off the agar plates, suspended in 100 µl sterile water and lysed by repeated shock-freezing in liquid nitrogen and thawing at 65 °C and finally centrifuged. An aliquot (1–3 µl) of the supernatant was used as template DNA for PCR amplification.

RAPD-PCR was performed using RAPD primers 1–6 of the RAPD Analysis primer set according to the instructions of the manufacturer (Pharmacia Biotech). The primers are here designated R1–R6. The different repetitive sequence PCR procedures (BOX-, ERIC- and REP-PCR) were done as described previously (Louws *et al.*, 1994; Wieser & Busse, 2000). Primers for BOX-, ERIC- and REP-PCR were synthesized at the Service Department of the Vienna Biocenter (MIG-BASE).

Amplified rDNA restriction analysis (ARDRA). 16S rRNA gene fragments amplified using the universal primers 27f and 1492r (Lane, 1991) were digested with the restriction enzymes *Hha*I and *Hin*II as recommended by the manufacturer (Promega). The digested DNA fragments were analysed by electrophoresis on a 4% NuSieve gel (FMC BioProducts), stained with ethidium bromide and photographed. The sizes of digested DNA fragments were estimated from migration distances of molecular size standards.

16S rDNA sequence analysis. Nearly the complete 16S rRNA gene of I/26-Cor1^T was amplified by PCR using the universal primers 27f and 1492r (Lane, 1991) as described previously (Wieser *et al.*, 1999). Fluorescently labelled primers 27f, 530f and 926f (Lane, 1991) were used for sequencing. Similarity searches with the derived sequence were done in the EMBL database using the program FASTA (Pearson & Lipman, 1988). The sequence was aligned with the most similar sequences from the database by using the program PILEUP (Devereux *et al.*, 1984). The sequence alignment was corrected manually and bases at the 5' and 3' ends were omitted from the analysis, resulting in 1248 bases (positions 118–1366, *Escherichia coli* numbering) that were included in the comparisons. Evolutionary distances (Jukes & Cantor, 1969) were calculated from nearly complete sequence-pair dissimilarities. A phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) with the program NEIGHBOR. The stability of the groupings was estimated by bootstrap analysis (1000 replications) using the software programs of the PHYLIP package (Felsenstein, 1993). All software programs were taken from the GCG programs (Genetics Computer Group, 1995).

RESULTS

Morphological and cultural characteristics

Cells of strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 were rod-shaped and 0.4–0.75 µm wide and 1.3–5.0 µm long. All three strains stained Gram-negative, showed Gram-negative behaviour after treatment with 3% KOH and were aminopeptidase-positive. No motility was observed by phase-contrast microscopy. Cells grew well on low-nutrient media

such as CasMM agar and R2A agar and good growth was observed on PYES agar. No growth was found on MacConkey agar or Czapek–Dox agar. Visible growth appeared on PYES agar after 3 d of inoculation. The colonies reached maximum size within 2 weeks. Isolates I/26-Cor1^T and I/32A-Cor1 produced slimy, irregular, pinkish-red colonies that spread on CasMM agar. Strain I/74-Cor2 did not spread on any agar tested.

Physiological and biochemical characteristics

The three strains were able to grow on PYES agar at temperatures from 4 to 28 °C and the best growth was observed at room temperature. At 4 °C, visible growth was observed after 16 d of incubation, and no growth was observed at 37 °C. The three strains grew without additional NaCl and in the presence of 0.5, 1.0 and 2.0% NaCl. No growth was observed in the presence of 4.0 or 10.0% NaCl after 12 d of incubation at room temperature. Haemolysis on blood agar was not observed. They were shown to produce catalase. Using the Bactident-Oxidase test, strains I/26-Cor1^T and I/32A-Cor1 were negative and strain I/74-Cor2 was weakly positive. Using the more sensitive Kovac's reagent for detection of oxidase activity, I/74-Cor2 was positive and strains I/26-Cor1^T and I/32A-Cor1 were weakly positive. An overall high similarity was detected in more than 70 physiological/biochemical characteristics of I/26-Cor1^T and I/32A-Cor1 but they could be distinguished readily from *Hymenobacter roseosalivarius* DSM 11622^T (different in nine traits), *Hymenobacter actinosclerus* CCUG 39621^T (different in 24 traits) and I/74-Cor2 (different in 13 traits) (Table 1).

Susceptibility to antimicrobials

Strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2, as well as *Hymenobacter actinosclerus* CCUG 39621^T, were susceptible to the action of bacitracin, chloramphenicol, colistin sulfate, erythromycin, fusidic acid, gentamicin, kanamycin, nitrofurantoin, penicillin G, polymyxin B, tetracycline and vancomycin.

Chemotaxonomic characteristics

The quinone systems of strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 were similar, consisting of the major menaquinone MK-7 (87–95%) with MK-6 (5–13%) present in minor amounts. The characteristic cell-wall diamino acid of all three strains was meso-diaminopimelic acid. Predominant fatty acids were C15:0 anteiso, C15:0 iso and summed feature 4 (16:1ω7c and/or 15:0 iso 2OH). All strains contained the hydroxy acids C15:0 iso 3OH, C15:0 2OH and C17:0 iso 3OH (Table 2). Strain I/74-Cor2 was clearly distinguished from the other two strains by the appreci-

Table 1. Biochemical characteristics of isolates I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2, *Hymenobacter actinosclerus* CCUG 39621^T and *Hymenobacter roseosalivarius* DSM 11622^T

Data for *Hymenobacter actinosclerus* CCUG 39621^T (physiological characteristics and API ZYM reactions) and *Hymenobacter roseosalivarius* DSM 11622^T (physiological characteristics and casein hydrolysis) were respectively obtained from Collins *et al.* (2000) and Hirsch *et al.* (1998). +, Positive; (+), weakly positive; –, negative; ND, not determined. All of the *Hymenobacter* strains showed weak assimilation of acetate and propionate and weak hydrolysis of *p*-nitrophenyl (pNP) α -D-glucopyranoside, bis-pNP phosphate, L-proline *p*-nitroanilide (pNA), L-alanine pNA and 2-deoxythymidine-5'-pNP phosphate. All of the *Hymenobacter* strains were negative for assimilation of L-arabinose, D-galactose, D-xylose, adonitol, *i*-inositol, D-sorbitol, putrescine, 4-aminobutyrate, azelate, β -alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate and for hydrolysis of pNP β -D-galactopyranoside, pNP β -D-glucuronide and pNP phosphorylcholine. In the API ZYM tests, all three airborne isolates were positive for alkaline phosphatase, esterase (C4), esterase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and for hydrolysis of DNA, starch and Tween 80. All three airborne isolates were negative in API ZYM tests for lipase (C14), trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase and nitrate reduction, urease (Christensen), citrate utilization (Simmons'), indole production and H₂S production.

Test	I/26-Cor1 ^T	I/32A-Cor1	I/74-Cor2	<i>H. actinosclerus</i> CCUG 39621 ^T	<i>H. roseosalivarius</i> DSM 11622 ^T
Growth at:					
4 °C	+	+	+	ND	+
5 °C	ND	ND	ND	–	+
28 °C	+	+	+	ND	–
32 °C	ND	ND	ND	ND	–
37 °C	–	–	–	ND	ND
42 °C	ND	ND	ND	+	ND
Oxidase:					
Bactident-Oxidase test	–	–	(+)	ND	ND
Kovac's reagent	(+)	(+)	+	+	+
Assimilation of:					
<i>N</i> -Acetyl D-glucosamine	–	–	–	(+)	–
<i>p</i> -Arbutin	–	–	–	(+)	–
D-Cellobiose	–	–	–	(+)	–
D-Fructose	(+)	(+)	–	–	–
Gluconate	–	–	–	(+)	–
D-Glucose	(+)	(+)	(+)	(+)	–
D-Mannose	(+)	(+)	(+)	(+)	–
D-Maltose	–	–	(+)	–	–
L-Rhamnose	–	–	(+)	–	–
D-Ribose	–	–	–	(+)	–
Sucrose	(+)	(+)	(+)	–	–
Salicin	–	–	(+)	–	–
D-Trehalose	–	–	(+)	–	–
Maltitol	–	–	(+)	–	–
D-Mannitol	–	–	(+)	–	–
<i>cis</i> -Aconitate	–	–	–	(+)	–
Adipate	–	–	–	(+)	–
Citrate	–	–	–	(+)	–
Fumarate	–	–	–	(+)	–
Glutarate	–	–	–	(+)	(+)
DL-3-Hydroxybutyrate	–	–	–	(+)	–
Itaconate	–	–	–	(+)	–
DL-Lactate	–	–	–	(+)	–
L-Malate	–	–	–	(+)	–
Mesaconate	–	–	–	(+)	–
Oxoglutarate	–	–	–	(+)	–
Pyruvate	–	–	–	(+)	(+)
Suberate	–	–	–	(+)	–
L-Alanine	–	–	–	(+)	–

Table 1 (cont.)

Test	I/26-Cor1 ^T	I/32A-Cor1	I/74-Cor2	<i>H. actinosclerus</i> CCUG 39621 ^T	<i>H. roseosalivarius</i> DSM 11622 ^T
L-Phenylalanine	–	–	–	(+)	–
L-Proline	–	–	–	(+)	–
Hydrolysis of:					
Casein	–	+	–	ND	+
Aesculin	–	–	(+)	–	–
pNP phenylphosphonate	(+)	(+)	(+)	(+)	–
pNP β-D-glucopyranoside	–	–	(+)	–	–
pNP β-D-xyloside	–	–	(+)	ND	ND
L-Glutamate γ-3-carboxy-pNA	(+)	(+)	(+)	(+)	–
N-Acetyl β-glucosaminidase (API ZYM)	–	–	+	ND	ND
Cystine arylamidase (API ZYM)	–	–	–	+	ND
Valine arylamidase (API ZYM)	+	+	–	+	ND

ably greater amount of 16:1ω5c and small amounts of summed feature 5 (17:1 anteiso B/iso I). *Hymenobacter actinosclerus* CCUG 39621^T could be distinguished from the three isolates on the basis of significant differences in the contents of C15:0 iso, summed feature 4 and 16:1ω5c (Table 2).

Phosphatidylethanolamine was found to be predominant in polar-lipid extracts of strains I/26-Cor1^T (Fig. 1a), I/32A-Cor1 and I/74-Cor2 (Fig. 1b). In addition, an unknown aminolipid and three unknown lipids (L1, L2, L5) were detected in all three strains. I/26-Cor1^T and I/32A-Cor1 were distinguished from each other by the absence of the unknown lipid L4 in I/32A-Cor1. I/74-Cor2 differed from the other two strains by the presence of the unknown lipid L3 and the absence of the unknown lipids L6 and L7. *Hymenobacter actinosclerus* CCUG 39621^T could be distinguished from the three isolates by the absence of the unidentified lipid L1. Diphosphatidylglycerol, which was reported in extracts from *Hymenobacter roseosalivarius* DSM 11622^T (Hirsch *et al.*, 1998), was not detected in the three strains or in *Hymenobacter actinosclerus* CCUG 39621^T. The polyamine pattern contained the predominant compound *sym*-homospermidine. The visible absorption spectrum of the acetone-extracted red pigment showed a maximum at 482 nm and two slight inflexions at 453 nm and 505 nm. The individual chemotaxonomic characteristics of strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 are summarized in Table 3.

G + C content of genomic DNA

Strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 had genomic DNA G+C contents of 63.1, 60.4 and 63.0 mol %.

Phylogenetic analyses

Analysis of the 16S rRNA gene of strain I/26-Cor1^T resulted in a sequence of 1362 bases. Comparison with corresponding sequences showed that strain I/26-Cor1^T shared the highest similarity with strains of ‘*Taxeobacter*’ (90.3–93.4%), *Hymenobacter roseosalivarius* DSM 11622^T (92.3%) and *Hymenobacter actinosclerus* CCUG 39621^T (95.8%). The sequence similarities to other members of the *Cytophaga–Flavobacterium–Bacteroides* group ranged from 79 to 83%. The phylogenetic analysis clearly demonstrated that strain I/26-Cor1^T is most closely related to strains of ‘*Taxeobacter*’ and *Hymenobacter* (Fig. 2).

Genomic fingerprints and ARDRA

Band patterns obtained after RAPD-PCR with primers R1–R6 demonstrated that isolates I/26-Cor1^T and I/32A-Cor2 cannot be distinguished using RAPD primers R1, R2, R3 and R6 (Fig. 3a, b) due to the homogeneity of their profiles. Clearly distinguishable genomic fingerprints for I/26-Cor1^T and I/32A-Cor2 were observed with primers R4 and R5 (Fig. 3b). In contrast, strain I/74-Cor2 displayed unique band patterns with all six RAPD primers (Fig. 3a, b). Genomic fingerprints generated after BOX-, ERIC- and REP-PCR were identical for isolates I/26-Cor1^T and I/32A-Cor1 (Fig. 4). The third strain, I/74-Cor2, and *Hymenobacter actinosclerus* CCUG 39621^T exhibited distinct genomic fingerprints with all three repetitive sequence PCR analyses, demonstrating their genomic distinctiveness.

When the PCR-amplified 16S rRNA genes from the three isolates were subjected to restriction endonuclease analysis using the enzyme *Hha*I (Fig. 5), identical band patterns were obtained for strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2. After digestion of the amplified 16S rDNA with the enzyme *Hinf*I, strains

Table 2. Major fatty acids of strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2, *Hymenobacter actinosclerus* CCUG 39621^T and *Hymenobacter roseosalivarius* DSM 11622^T

Values are percentages of total fatty acids. Strains I/26-Cor1^T, I/32A-Cor1, I/74-Cor2 and *H. actinosclerus* CCUG 39621^T were grown on TSA for 72 h at 28 °C prior to analysis. *H. roseosalivarius* DSM 11622^T was grown on R2A agar at 28 °C for 72 h prior to analysis. 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* isomers are indicated by the suffix *c*. Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contains one or more of the following fatty acids: 15:1 iso I and 13:0 3OH. Summed feature 4 contains one or more of the following fatty acids: 16:1 ω 7*c* and 15:0 iso 2OH. Summed feature 5 contains one or more of the following fatty acids: 17:1 anteiso B/iso I.

Compound	I/26-Cor1 ^T	I/32A-Cor1	I/74-Cor2	<i>H. actinosclerus</i> CCUG 39621 ^T	<i>H. roseosalivarius</i> DSM 11622 ^T
Unknown 13:566	–	–	0.4	–	–
14:0	–	–	0.5	–	–
14:0 iso	–	0.4	–	–	–
Summed feature 2	0.8	1.4	0.6	2.3	–
15:1 anteiso A	–	0.5	–	–	–
15:0 iso	10.8	15.0	16.8	22.3	8.3
15:0 anteiso	22.3	18.6	13.3	25.8	–
15:1 ω 6 <i>c</i>	0.8	1.2	0.5	0.8	–
16:1 iso H	1.5	1.4	0.9	1.5	2.7
16:1 ω 7 <i>c</i> alcohol	–	–	–	–	0.7
16:0 iso	0.8	0.8	0.4	–	2.1
16:0 anteiso	–	0.7	–	–	–
Summed feature 4	21.4	22.3	19.3	13.1	29.8
16:1 ω 5 <i>c</i>	7.9	6.6	17.4	3.7	23.3
16:0	1.7	1.6	4.8	–	1.1
15:0 iso 3OH	1.6	1.3	1.5	1.6	2.7
15:0 2OH	0.9	0.5	0.4	0.8	–
Summed feature 5	17.7	18.8	10.1	19.9	18.5
Unknown 16:580	–	–	0.4	–	–
iso 17:1 ω 9 <i>c</i>	–	0.5	–	–	–
17:0 iso	4.5	2.7	6.6	1.8	1.7
17:0 anteiso	2.3	1.3	1.0	0.7	–
17:1 ω 6 <i>c</i>	–	0.7	–	0.7	1.1
16:0 iso 3OH	–	–	–	–	1.0
16:0 3OH	–	–	–	–	1.2
17:0 iso 3OH	3.5	2.6	3.6	3.1	5.8
17:0 2OH	1.3	0.8	–	2.0	–

I/26-Cor1^T and I/32A-Cor1 were not distinguishable from each other. The sizes of the single fragments were in excellent agreement with those obtained when the 16S rDNA sequence of I/26-Cor1^T was theoretically digested, resulting in fragments of 875, 321, 107 and 59 bp (*Hha*I) and 487, 266, 222, 165, 149, 45, 15 and 13 bp (*Hinf*I). The discrepancy between the presence of a band in the *Hinf*I pattern at approximately 320 bp (Fig. 5) and the theoretical band of 222 bp can be easily explained by the fact that the stretch of the 16S rDNA that was subjected to digestion was obtained after amplification with the primer pair 27f/1492r whereas, in the sequence used for the theoretical digest, the first 90 nucleotides were missing. Employing this theoretical approach, *Hymenobacter actinosclerus* CCUG 39621^T (accession no. Y17356) was charac-

terized by the same band patterns, indicating a close relationship, as shown already by comparison of almost complete 16S rDNA sequences. Strain I/74-Cor2 was slightly different from the other two strains in lacking bands at 270 and 50 bp in the *Hinf*I pattern (Fig. 5). This observation might be explained by their digestion into smaller bands that were not detectable on the 4% NuSieve agarose gel or by the presence of two bands of approximately the same size that could not be distinguished in the gel. Employing the two enzymes, the theoretical digestion patterns of *Hymenobacter roseosalivarius* DSM 11622^T (accession no. Y18833), which is more distantly related, showed greater differences from the other strains than the patterns of strain I/74-Cor2. Thus, the *Hinf*I pattern of strain I/74-Cor2 indicates that it is a member of the

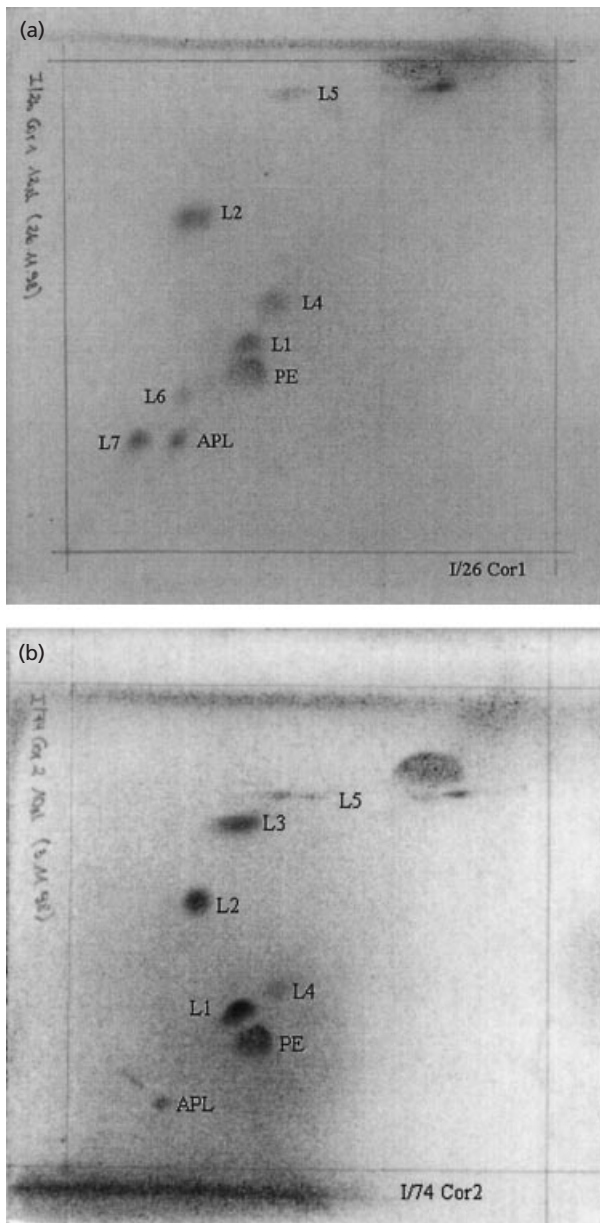


Fig. 1. Two-dimensional TLC of polar lipid extracts obtained from strains I/26-Cor1^T (a) and I/74-Cor2 (b) stained with molybdatophosphoric acid. PE, Phosphatidylethanolamine; APL, unknown aminophospholipid; L1–L7, unknown polar lipids.

genus *Hymenobacter* that is related more closely to *Hymenobacter actinosclerus* CCUG 39621^T than to *Hymenobacter roseosalivarius* DSM 11622^T.

DISCUSSION

Generally, maximum death rates for airborne bacterial cells occur in the first 0.7–1.0 s after aerosolization (Stetzenbach, 1992). Since they were isolated from air, isolates I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 can be assumed to be resistant to desiccation. This property

would support their survival when suspended in the air. Desiccation resistance has been reported for strains of ‘*Taxeobacter*’ isolated from air-dried samples that had been stored for more than 5 years at room temperature (Reichenbach, 1992). Also, *Hymenobacter roseosalivarius* DSM 11622^T was isolated from a very dry environment (Hirsch *et al.*, 1998). Desiccation resistance might also be assumed for the close relative *Hymenobacter actinosclerus* CCUG 39621^T (Collins *et al.*, 2000). This species was described as being resistant to high levels of radiation. A correspondence between adaptation to dehydration and radiation resistance was assumed previously for *Deinococcus radiodurans* (Mattimore & Battista, 1996). If a similar mechanism is present in *Hymenobacter actinosclerus* CCUG 39621^T, this species might also be resistant to desiccation and this characteristic might be common to the members of the ‘*Taxeobacter*’–*Hymenobacter* branch.

Our phylogenetic analysis demonstrated that isolate I/26-Cor1^T is related most closely to ‘*Taxeobacter*’ strains, *Hymenobacter roseosalivarius* strains DSM 11622^T and DSM 11621 (Hirsch *et al.*, 1998) and *Hymenobacter actinosclerus* CCUG 39621^T (Collins *et al.*, 2000). The genomic relatedness to these taxa is supported by its similar chemotaxonomic properties such as the quinone composition and the presence of characteristic polar lipids as well as the high G+C content of the genomic DNA (Table 3; Hirsch *et al.*, 1998). The latter has not been reported for any other taxon within the *Cytophaga*–*Flavobacterium*–*Bacteroides* group (Reichenbach, 1989, 1992; Bernardet *et al.*, 1996; Nakagawa & Yamasato, 1996; Nakagawa *et al.*, 1997; Hirsch *et al.*, 1998; Collins *et al.*, 2000). Despite similarities in chemotaxonomic properties to ‘*Taxeobacter*’ Txcl, ‘*Taxeobacter*’ Txlgl, *Hymenobacter roseosalivarius* (Hirsch *et al.*, 1998) and *Hymenobacter actinosclerus* CCUG 39621^T, the significant divergence in the 16S rDNA sequence (90.3–95.8% similarity) demonstrates clearly that I/26-Cor1^T represents an undescribed species of the genus *Hymenobacter* within the *Cytophaga*–*Flavobacterium*–*Bacteroides* group. Identical ARDRA patterns demonstrated a closer relatedness between I/26-Cor1^T and I/32A-Cor1. These patterns were also identical to those obtained after theoretical digestion of the rDNA of *Hymenobacter actinosclerus* CCUG 39621^T, demonstrating the close relatedness of the three strains. Strain I/74-Cor2 shared an identical *Hha*I ARDRA pattern with the other two isolates but differences were observed in the *Hinf*I pattern. However, the ARDRA patterns of I/74-Cor2 showed greater similarities to the corresponding patterns of the other two isolates and *Hymenobacter actinosclerus* CCUG 39621^T than to those that would be expected after theoretical digestion of the rDNA of *Hymenobacter roseosalivarius* DSM 11622^T. Based on the conserved structure of rDNA, it can be concluded that I/74-Cor2 is another member of the genus *Hymenobacter* that is closely related to I/26-Cor1^T,

Table 3. Chemotaxonomic characteristics of strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2, *Hymenobacter actinosclerus* CCUG 39621^T and *Hymenobacter roseosalivarius* DSM 11622^T

All strains listed have MK-7 as the major menaquinone. Abbreviations: PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; APL, unknown aminophospholipid; L1–L7, unknown polar lipids; Hspd, *sym*-homospermidine; *m*-DAP, *meso*-diaminopimelic acid.

Characteristic	I/26-Cor1 ^T	I/32A-Cor1	I/74-Cor2	<i>H. actinosclerus</i> CCUG 39621 ^T	<i>H. roseosalivarius</i> DSM 11622 ^T
Polar lipids	PE, APL, L1, L2, L4–L7	PE, APL, L1, L2, L5–L7	PE, APL, L1–L5	PE, APL, L2, L3, L5–L7	PE, DPG ^{b*}
Cell-wall diamino acid	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP ^a	ND
Major polyamine	Hspd	Hspd	Hspd	ND	ND
G + C content (mol%)	63	60	63	62 ^a	56 ^b

* Data taken from Collins *et al.* (2000) (a) or Hirsch *et al.* (1998) (b).

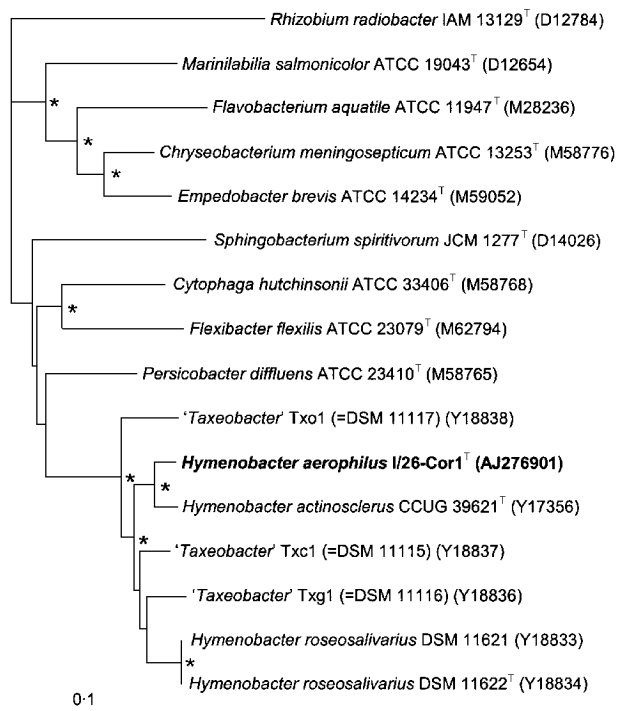


Fig. 2. Dendrogram indicating the estimated phylogenetic relationship between strain I/26-Cor1^T and other members of the *Cytophaga*–*Flavobacterium*–*Bacteroides* group based on a comparison of 16S rDNA sequences. Branching points that displayed significant bootstrap values (> 75%) are indicated with asterisks. Sequence data for other strains were obtained from the EMBL database. *Rhizobium radiobacter* IAM 13129^T was included as an outgroup.

I/32A-Cor1 and *Hymenobacter actinosclerus* CCUG 39621^T.

The close relatedness of the three red-pigmented, airborne bacterial strains I/26-Cor1^T, I/32A-Cor1 and I/74-Cor2 is also demonstrated by the striking similarities in their quinone systems, polyamine patterns, polar lipid compositions and fatty acids. I/26-Cor1^T

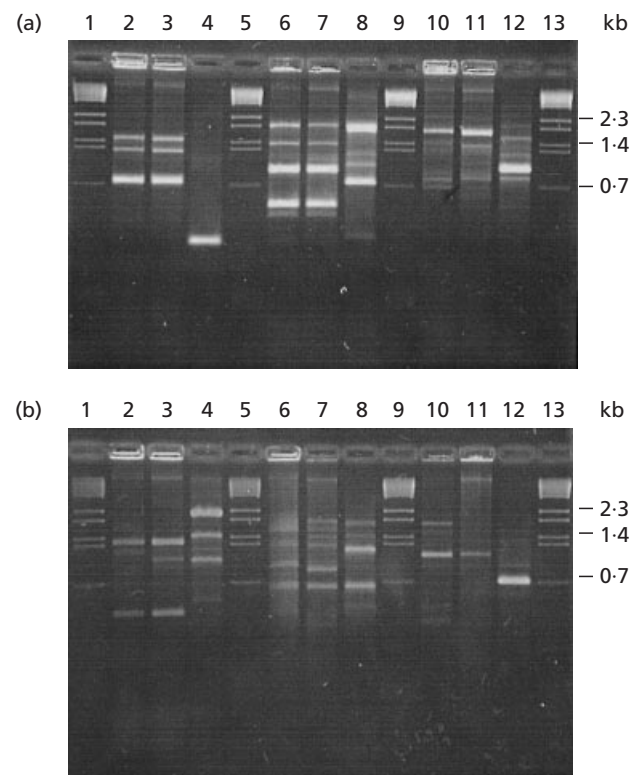


Fig. 3. Genomic fingerprints of the three red-pigmented, airborne bacterial isolates generated by RAPD-PCR. (a) Fingerprints generated using primers R1 (lanes 2–4), R2 (6–8) and R3 (10–12). Lanes 1, 5, 9 and 13, lambda DNA *Bst*EII digest; 2, 6 and 10, I/26-Cor1^T DNA; 3, 7 and 11, I/32A-Cor1 DNA; 4, 8 and 12, I/74-Cor2 DNA. (b) Fingerprints generated using primers R4 (lanes 2–4), R5 (6–8) and R6 (10–12). Lanes 1, 5, 9 and 13, lambda DNA *Bst*EII digest; 2, 6 and 10: I/26-Cor1^T DNA; 3, 7 and 11, I/32A-Cor1 DNA; 4, 8 and 12, I/74-Cor2 DNA.

and I/32A-Cor1 could be distinguished from each other only by the presence of an unidentified polar lipid, some small quantitative differences in certain fatty acids, the hydrolysis of casein, the G + C content of genomic DNA and band patterns generated after

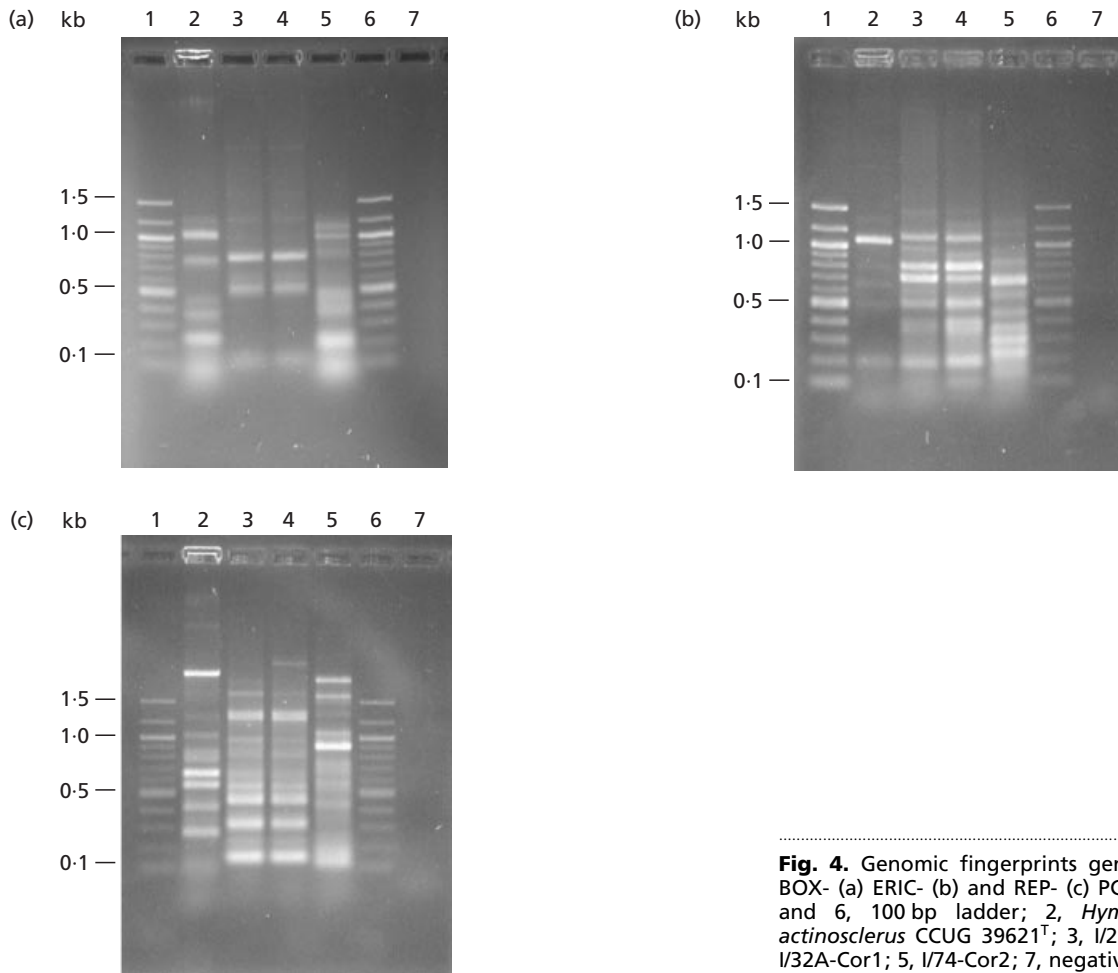


Fig. 4. Genomic fingerprints generated by BOX- (a) ERIC- (b) and REP- (c) PCR. Lanes 1 and 6, 100 bp ladder; 2, *Hymenobacter actinosclerus* CCUG 39621^T; 3, I/26-Cor1^T; 4, I/32A-Cor1; 5, I/74-Cor2; 7, negative control.

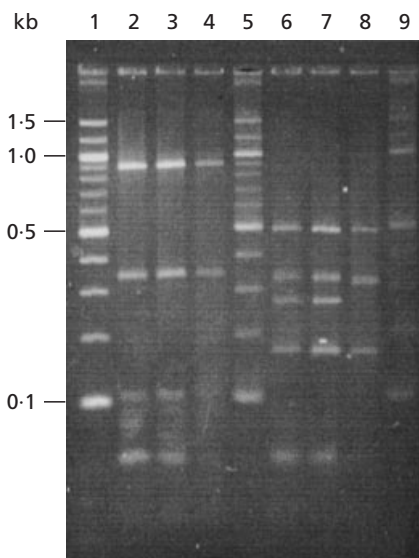


Fig. 5. ARDRA analysis of 16S rRNA genes of the three airborne bacteria amplified with the universal eubacterial primers 27f and 1492r and digested with *Hha*I (lane 2, I/26-Cor1^T; 3, I/32A-Cor1; 4, I/74-Cor2) or *Hinf*I (lane 6, I/26-Cor1^T; 7, I/32A-Cor1; 8, I/74-Cor2) and separated in a 4% NuSieve gel. Lanes 1, 5 and 9, 100 bp marker.

RAPD-PCR using primers R4 and R5 (Fig. 3b). Almost identical biochemical profiles (Table 1) and identical band patterns generated after PCR analyses using BOX, ERIC and REP primers (Fig. 4) and some RAPD primers (primers R1, R2, R3 and R6; Fig. 3) indicate the clonal relatedness of strains I/26-Cor1^T and I/32A-Cor1 and they can therefore be considered as members of a single species. I/74-Cor2 could be distinguished from I/26-Cor1^T and I/32A-Cor1 by obvious dissimilarities in the band patterns generated after the different PCR-based analyses (Figs 3 and 4) and quantitative differences in the fatty acid profiles (Table 2) and polar lipid profiles (Table 3). The chemotaxonomic characteristics, as well as the similarity in the ARDRA profiles and the high G+C content of genomic DNA, confirm the affiliation of I/74-Cor2 to the genus *Hymenobacter* but the observed quantitative differences in the fatty acid profile and the differences in the *Hinf*I ARDRA pattern indicate that, most likely, strain I/74-Cor2 represents another species.

The three airborne isolates can be distinguished from *Hymenobacter roseosalivarius* by differences in the fatty acid profile and the polar lipid composition. The two strains of *Hymenobacter roseosalivarius* were reported to contain only traces of C15:0 anteiso

(Hirsch *et al.*, 1998) and, in our reanalysis of the fatty acid profile of *Hymenobacter roseosalivarius* DSM 11622^T, this fatty acid was absent from the profile (Table 2). In the three airborne isolates, C15:0 anteiso was present in large amounts (Table 2). Additionally, significant quantitative differences in certain fatty acids, such as C15:0 iso and C16:1 ω 7c, clearly distinguish the airborne isolates from the two strains of *Hymenobacter roseosalivarius* (Hirsch *et al.*, 1998). The airborne isolates I/26-Cor1^T and I/32A-Cor1 could be distinguished from *Hymenobacter actinoscleris* CCUG 39621^T by the absence of one polar lipid (L1) and the presence of another lipid (L3), quantitative differences in the major fatty acids (Table 2), differences in relation to growth temperature and numerous biochemical traits (Table 1; Collins *et al.*, 2000).

Based on the low degree of 16S rRNA gene sequence similarity of strain I/26-Cor1^T to established species (Fig. 2) and the high similarity of the majority of analysed characteristics of I/26-Cor1^T and I/32A-Cor1, it is obvious that they represent a novel species of the genus *Hymenobacter*. Consequently, I/26-Cor1^T and I/32A-Cor1 are described in this report as a novel species, for which the name *Hymenobacter aerophilus* sp. nov. is proposed. It is most likely that strain I/74-Cor2 represents another species of the genus *Hymenobacter*, as indicated by its distinct biochemical traits (Table 1), fatty acid profile (Table 2) and ARDRA profile (Fig. 5) and its genomic fingerprints (Figs 3–4). However, a distinct species cannot be definitively described for strain I/74-Cor2, since its taxonomic position within the genus *Hymenobacter* has not been demonstrated unambiguously.

Description of *Hymenobacter aerophilus* sp. nov.

Hymenobacter aerophilus (aer.o.phi'lus. N.L. adj. *aerophilus* lover of air, indicating its survival when suspended in the air).

Cells are rod-shaped, Gram-negative, aerobic, non-spore-forming bacteria. Motility is not observed. Cells are 0.4–0.75 μ m wide and 1.3–5.0 μ m long. Cells grow best on nutrient-reduced media such as CasMM and R2A agar. Colonies on CasMM and R2A agar are translucent, red, circular, entire, low-convex, smooth and slimy; diameter is up to 3.0 mm after 5 d at 28 °C and they spread on CasMM agar. Colonies on standard bacteriological media such as PYES agar and TSA are opaque, red, circular, entire, convex and smooth; diameter is up to 2.0 mm after 5 d of incubation at 28 °C. No growth occurs on MacConkey or Czapek–Dox agar. The optimum growth temperature is room temperature. The temperature range for growth is 4–28 °C; no growth at 37 °C. Tolerance maximum for growth in the presence of NaCl is 2.0%. MK-7 is the predominant menaquinone and meso-diaminopimelic acid is the diagnostic diamino acid in

the cell wall. Phosphatidylethanolamine is the only known lipid in the polar-lipid extract. Major cellular fatty acids are C15:0 anteiso, C15:0 iso and C16:1. Water-insoluble red pigment is produced; the visible absorption spectrum of the acetone-extracted pigment shows a maximum at 482 nm and two slight inflexions at 453 nm and 505 nm. Cells assimilate acetate, propionate, D-fructose, D-glucose, D-mannose and sucrose and display positive reactions in the following tests: catalase, L-alanine aminopeptidase, DNase, alkaline phosphatase, esterase (C4), esterase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, hydrolysis of Tween 80, *p*-nitrophenyl (pNP) α -D-glucopyranoside, pNP phenylphosphonate, L-proline *p*-nitroanilide (pNA), L-alanine pNA, L-glutamate γ -3-carboxy-pNA, bis-pNP phosphate and 2-deoxythymidine-5'-pNP phosphate. Depending on the sensitivity of the method applied, strains show no or only a weakly positive oxidase reaction. Other reactions in which strains show variable or negative results are listed in Table 1. Cells are susceptible to bacitracin, chloramphenicol, colistin sulfate, erythromycin, fusidic acid, gentamicin, kanamycin, nitrofurantoin, penicillin G, polymyxin B, tetracycline and vancomycin. Isolated from the air in the Museo Correr, Venice, Italy. DNA base composition ranges from 60 to 63 mol% (HPLC). Strain I/26-Cor1^T was determined to have a G+C content of 63.1 mol%.

The type strain is I/26-Cor1^T (= DSM 13606^T = LMG 19657^T). Another strain is I/32A-Cor1 (= DSM 13607 = LMG 19658).

ACKNOWLEDGEMENTS

This paper is dedicated to Karlheinz Altendorf (Osnabrück) on the occasion of his 60th birthday. We would like to acknowledge the support of the Project AER from the European Commission in the R&D Programme Environment and Climate, contract no. ENV4-CT95-088. We are grateful to Michelle Glew for critical reading of the manuscript.

REFERENCES

- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.
- Auling, G., Probst, A. & Kroppenstedt, R. M. (1986). Chemo- and molecular taxonomy of D(-)-tartrate-utilizing pseudomonads. *Syst Appl Microbiol* **8**, 114–120.
- Bauwens, M. & De Ley, J. (1981). Improvements in the taxonomy of *Flavobacterium* by DNA:rRNA hybridization. In *The Flavobacterium-Cytophaga Group* (GBF Monograph Series no. 5), pp. 27–31. Edited by H. Reichenbach & O. B. Weeks. Weinheim: Verlag Chemie.
- Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.

- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997).** *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- Bowman, J. P., McCammon, S. A., Lewis, T., Skerratt, J. H., Brown, J. L., Nichols, D. S. & McMeekin, T. A. (1998).** *Psychroflexus torquus* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**, 1601–1609.
- Busse, H. J. & Auling, G. (1988).** Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997).** Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* **47**, 698–708.
- Camuffo, D., Brimblecombe, P., Van Grieken, R. & 10 other authors (1999).** Indoor air quality at the Correr Museum, Venice, Italy. *Sci Total Environ* **236**, 135–152.
- Collins, M. D., Hutson, R. A., Grant, I. R. & Patterson, M. F. (2000).** Phylogenetic characterization of a novel radiation-resistant bacterium from irradiated pork: description of *Hymenobacter actinosclerus* sp. nov. *Int J Syst Evol Microbiol* **50**, 731–734.
- Devereux, J., Haerberli, P. & Smithies, O. (1984).** A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Felsenstein, J. (1993).** PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, WA, USA.
- Genetics Computer Group (1995).** *GCG Package Version 8.1 Program Manual*. Madison, WI: Genetics Computer Group.
- Gosink, J. J., Woese, C. R. & Staley, J. T. (1998).** *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga*–*Flavobacterium*–*Bacteroides* group and reclassification of ‘*Flectobacillus glomeratus*’ as *Polaribacter glomeratus* comb. nov. *Int J Syst Bacteriol* **48**, 223–235.
- Hirsch, P., Ludwig, W., Hethke, C., Sittig, M., Hoffmann, B. & Gallikowski, C. A. (1998).** *Hymenobacter roseosalivarius* gen. nov., sp. nov. from continental Antarctic soils and sandstone: bacteria of the *Cytophaga*/*Flavobacterium*/*Bacteroides* line of phylogenetic descent. *Syst Appl Microbiol* **21**, 374–383.
- Holmes, B., Steigerwalt, A. G., Weaver, R. E. & Brenner, D. J. (1986).** *Weeksella virosa* gen. nov., sp. nov. (formerly group IIF), found in human clinical specimens. *Syst Appl Microbiol* **8**, 185–190.
- Jukes, T. H. & Cantor, C. R. (1969).** Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P., Steiof, M. & Dott, W. (1991).** Microbiological characterisation of a fuel oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Denner, E. B. M., Meyer, S., Moore, E. R. B. & Busse, H.-J. (1997).** Classification of ‘*Pseudomonas azotocolligans*’ Anderson 1955, 132, in the genus *Sphingomonas* as *Sphingomonas trueperi* sp. nov. *Int J Syst Bacteriol* **47**, 577–583.
- Kaneko, T., Katoh, K., Fujimoto, M., Kumagi, M., Tamaoka, J. & Katayama-Fujimura, Y. (1986).** Determination of the nucleotide composition of a deoxyribonucleic acid by high-performance liquid chromatography of its enzymatic hydrolysates: a review. *J Microbiol Methods* **4**, 229–240.
- Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Louws, F. J., Fulbright, D. W., Stephens, C. T. & de Bruijn, F. J. (1994).** Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Appl Environ Microbiol* **60**, 2286–2295.
- Mattimore, V. & Battista, J. R. (1996).** Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J Bacteriol* **178**, 633–637.
- Moaledji, K. (1986).** Comparison of Gram-staining and alternate methods, KOH test and aminopeptidase activity in aquatic bacteria: their application to numerical taxonomy. *J Microbiol Methods* **5**, 303–310.
- Nakagawa, Y. & Yamasato, K. (1993).** Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.
- Nakagawa, Y. & Yamasato, K. (1996).** Emendation of the genus *Cytophaga* and transfer of *Cytophaga agarovorans* and *Cytophaga salmonicolor* to *Marimilibia* gen. nov.: phylogenetic analysis of the *Flavobacterium*–*Cytophaga* complex. *Int J Syst Bacteriol* **46**, 599–603.
- Nakagawa, Y., Hamana, K., Sakane, T. & Yamasato, K. (1997).** Reclassification of *Cytophaga aprica* (Lewin 1969) Reichenbach 1989 in *Flammeovirga* gen. nov. as *Flammeovirga aprica* comb. nov. and of *Cytophaga diffluens* (ex Stanier 1940; emend. Lewin 1969) Reichenbach 1989 in *Persicobacter* gen. nov. as *Persicobacter diffluens* comb. nov. *Int J Syst Bacteriol* **47**, 220–223.
- Paster, B. J., Ludwig, W., Weisburg, W. G., Stackebrandt, E., Hespell, R. B., Hahn, C. M., Reichenbach, H., Stetter, K. O. & Woese, C. R. (1985).** A phylogenetic grouping of the bacteroides, cytophagas, and certain flavobacteria. *Syst Appl Microbiol* **6**, 34–42.
- Paster, B. J., Dewhirst, F. E., Olsen, I. & Fraser, G. J. (1994).** Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas* spp. and related bacteria. *J Bacteriol* **176**, 725–732.
- Pearson, W. R. & Lipman, D. J. (1988).** Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.
- Porter, K. & Feig, Y. S. (1990).** The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**, 943–948.
- Reichenbach, H. (1989).** Genus I. *Cytophaga* Winogradsky 1929. In *Bergey’s Manual of Systematic Bacteriology*, vol. 3, pp. 2015–2050. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Reichenbach, H. (1992).** The order *Cytophagales*. In *The Prokaryotes – a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Application*, 2nd edn, pp. 3631–3675. Edited by A. Balows, H.-G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. P. (1985).** Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Segers, P., Mannheim, W., Vancanneyt, M., De Brandt, K., Hinz, K.-H., Kersters, K. & Vandamme, P. (1993a).** *Riemerella anatipestifer* gen. nov., comb. nov., the causative agent of septicemia anserum exsudativa, and its phylogenetic affiliation within the *Flavobacterium*–*Cytophaga* rRNA homology group. *Int J Syst Bacteriol* **43**, 768–776.
- Segers, P., Vandamme, P., Steyn, P. L., Mannheim, W., Willekens, H., Bauwens, M., De Ley, J. & Kersters, K. (1993b).** Phylogenetic studies of *Flavobacterium* and related organisms by DNA–rRNA hybridizations. In *Advances in the Taxonomy and Significance of Flavobacterium, Cytophaga and Related Bacteria*, pp. 129–136. Edited by P. J. Jooste. Bloemfontein: University of the Orange Free State Press.
- Sherr, B. F., Sherr, E. B. & McDaniel, J. (1992).** Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. *Appl Environ Microbiol* **58**, 2381–2385.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterisation. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Woods & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stetzenbach, L. D. (1992).** Airborne microorganisms. In *Encyclopedia of Microbiology*, vol. 1, pp. 53–65. Edited by J. Lederberg. San Diego: Academic Press.

- Takeuchi, M. & Yokota, A. (1992).** Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalpophilum* comb. nov., and two genospecies of the genus *Sphingobacterium*, and synonymy of *Flavobacterium yabuuchiae* and *Sphingobacterium spiritivorum*. *J Gen Appl Microbiol* **38**, 465–482.
- Tindall, B. J. (1990).** A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Vandamme, P., Segers, P., Vancanneyt, M. & 11 other authors (1994a).** *Ornithobacterium rhinotracheale* gen. nov., sp. nov., isolated from the avian respiratory tract. *Int J Syst Bacteriol* **44**, 24–37.
- Vandamme, P., Bernardet, J.-F., Segers, P., Kersters, K. & Holmes, B. (1994b).** New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int J Syst Bacteriol* **44**, 827–831.
- Ventosa, A., Marquez, M. C., Kocur, M. & Tindall, B. J. (1993).** Comparative study of ‘*Micrococcus* sp.’ strains CCM 168 and CCM 1405 and members of the genus *Salinicoccus*. *Int J Syst Bacteriol* **43**, 245–248.
- Weisburg, W. G., Oyaizu, Y., Oyaizu, H. & Woese, C. R. (1985).** Natural relationship between bacteroides and flavobacteria. *J Bacteriol* **164**, 230–236.
- Wieser, M. & Busse, H.-J. (2000).** Rapid identification of *Staphylococcus epidermidis*. *Int J Syst Evol Microbiol* **50**, 1087–1093.
- Wieser, M., Schumann, P., Martin, K., Altenburger, P., Burghardt, J., Lubitz, W. & Busse, H.-J. (1999).** *Agrococcus citreus* sp. nov., isolated from a medieval wall painting of the chapel of Castle Herberstein (Austria). *Int J Syst Bacteriol* **49**, 1165–1170.
- Woese, C. R., Yang, D., Mandelco, L. & Stetter, K. O. (1990).** The *Flexibacter*–*Flavobacterium* connection. *Syst Appl Microbiol* **13**, 161–165.
- Yano, I., Tomiyasu, I. & Yabuuchi, E. (1982).** Long chain base composition of strains of three species of *Sphingobacterium* gen. nov. *FEMS Microbiol Lett* **15**, 303–307.