Pigmentiphaga daeguensis sp. nov., isolated from wastewater of a dye works, and emended description of the genus *Pigmentiphaga*

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A Gram-negative, non-spore-forming, rod-shaped *Pigmentiphaga*-like bacterial strain, K110^T, was isolated from wastewater collected from a dye works in Korea and was subjected to a polyphasic taxonomic analysis. Strain K110^T grew optimally at pH 7.0–8.0 and 37 °C in the presence of 0.5 % (w/v) NaCl. It contained Q-8 as the predominant ubiquinone and C_{16:0}, cyclo C_{17:0} and cyclo C_{19:0ω8c} as the major fatty acids. The major polar lipids were phosphatidylglycerol, phosphatidylethanolamine and two unidentified aminolipids. The DNA G + C content was 67.4 mol%. In a neighbour-joining phylogenetic tree constructed on the basis of 16S rRNA gene sequences, strain K110^T joined *Pigmentiphaga kullae*, the sole species of the genus, at a bootstrap confidence level of 100 %. Strain K110^T exhibited a 16S rRNA gene sequence similarity of 99.4 % with respect to the type strain of *P. kullae*. Although strain K110^T was found to be similar to *P. kullae* in terms of phenotypic properties, it differed in terms of motility, polar lipids, DNA–DNA relatedness and repetitive extragenic palindromic PCR genomic fingerprinting patterns. On the basis of phenotypic, phylogenetic and genetic data, strain K110^T represents a novel species of the genus *Pigmentiphaga*, for which the name *Pigmentiphaga daeguensis* sp. nov. is proposed. The type strain is K110^T (= KCTC 12838^T = JCM 14330^T).

The genus *Pigmentiphaga* was proposed by Blümel *et al.* (2001) with *Pigmentiphaga kullae* as the sole recognized species of the genus. Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Pigmentiphaga* falls within the *Betaproteobacteria* (Blümel *et al.*, 2001). In this study, we report on the taxonomic characterization of a *Pigmentiphaga*-like bacterial strain, K110^T, which was isolated from wastewater collected from a dye works.

Wastewater collected from a dye works at Daegu, Korea, was used as the source for the isolation of bacterial strains. Strain K110^T was isolated by means of the standard dilution plating technique on trypticase soy agar (TSA; Difco) at 30 °C. *P. kullae* DSM 13608^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The morphological, physiological and biochemical characteristics of strain K110^T were

investigated using routine cultivation on TSA at 37 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy with cells from exponentially growing cultures. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–50 °C) was measured on TSA. The pH range for growth was determined in nutrient broth (Difco) adjusted, prior to sterilization, to various pH values (pH 4.5–10.5, in increments of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth in the absence of NaCl and at various NaCl concentrations (0.5 %, w/v, and 1.0–10.0 %, w/v, at increments of 1.0 %) was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with nitrate, both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis

Abbreviation: rep-PCR, repetitive extragenic palindromic PCR.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K110^T is EF100696.

rep-PCR fingerprints and cellular fatty acid compositions for strain K110^T and *P. kullae* DSM 13608^T are available as supplementary material with the online version of this paper.

of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). DNase activity was examined by using DNase test agar with methyl green (Difco). Hydrolysis of aesculin and nitrate reduction were studied as described previously (Lanyi, 1987). Susceptibility to antibiotics was tested on TSA plates by using antibiotic discs containing the following amounts of antibiotic: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Utilization of various substrates, activities of various enzymes and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux); the cells were suspended in AUX medium, according to the manufacturer's instructions, to inoculate the API 50 CH system.

Cell biomass for DNA extraction and for isoprenoid quinone and polar lipid analyses was obtained from cultivation in trypticase soy broth (Difco) at 37 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by a PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain K110^T and *P. kullae* DSM 13608^T was harvested from TSA plates after incubation for 3 days at 37 °C. The fatty acids were extracted and the fatty acid methyl esters prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and then identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed and the resulting nucleotides analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means for the remaining three values are quoted as the DNA–DNA relatedness values. Repetitive extragenic palindromic PCR (rep-PCR) genomic fingerprinting using REP, BOX and (GTG)₅ PCR primers was performed as described by Rademaker *et al.* (1998). Computer-assisted analysis of the genomic fingerprints

was performed by using GelCompar II software, version 1.5 (Applied Maths).

Morphological, cultural, physiological and biochemical characteristics of strain K110^T are given in the species description (see below) or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain K110^T determined in this study comprised 1486 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analysis showed that strain K110^T was most closely related phylogenetically to the genus *Pigmentiphaga* (Fig. 1). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain K110^T joined the type strain of *P. kullae* at a bootstrap resampling value of 100 % (Fig. 1). Strain K110^T exhibited a 16S rRNA gene sequence similarity value of 99.4 % with respect to the type strain of *P. kullae* and showed less than 96.4 % similarity with respect to other species included in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain K110^T was Q-8 (peak area ratio, approx. 94 %). Strain

Table 1. Differential phenotypic characteristics of strain K110^T and *P. kullae* DSM 13608^T

Data for *P. kullae* DSM 13608^T were obtained from Blümel *et al.* (2001) and this study. Cells of both strains are Gram-negative, non-spore-forming rods. Both strains are positive for catalase, oxidase, hydrolysis of hypoxanthine, utilization of citrate, susceptibility to chloramphenicol, gentamicin, kanamycin, neomycin and polymyxin B, esterase (C4), esterase lipase (C8), leucine arylamidase, α -chymotrypsin and naphthol-AS-BI-phosphohydrolase (weakly). Both are negative for the following: utilization of L-arabinose, ribose, D-xylose, adonitol, galactose, glucose, fructose, mannose, rhamnose, inositol, mannitol, sorbitol, N-acetylglucosamine, arbutin, salicin, cellobiose, maltose, melibiose, sucrose, trehalose and gluconate, nitrate reduction, hydrolysis of aesculin, casein, gelatin, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine, susceptibility to ampicillin, cephalothin, lincomycin, novobiocin, oleandomycin, penicillin G and streptomycin and activity of alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Characteristic	Strain K110 ^T	<i>P. kullae</i> DSM 13608 ^T
Motility	—	+
Susceptibility to:		
Tetracycline	+	—
Carbenicillin	—	+
Major polar lipid(s)*	PG, PE, AL	PE
DNA G + C content (mol%)	67.4	68.5 ± 0.3 (67.9)†

*AL, Unidentified aminolipids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

†Data from this study for strain DSM 13608^T obtained using HPLC.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain K110^T and some related taxa. Bootstrap percentages (based on 1000 replications) greater than 50% are shown at branching points. *Burkholderia cepacia* ATCC 25416^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

K110^T had a cellular fatty acid profile that contained large amounts of straight-chain and hydroxy fatty acids; the major components (>10% of total fatty acids) were C_{16:0}, cyclo C_{17:0} and cyclo C_{19:0ω8c} (see Supplementary Table S1, available in IJSEM Online). This fatty acid profile was similar to that of the type strain of *P. kullae*, although there were differences in the proportions of some fatty acids (Supplementary Table S1). The major polar lipids detected in strain K110^T were phosphatidylglycerol, phosphatidylethanolamine and two unidentified aminolipids; minor amounts of diphosphatidylglycerol and an unidentified phospholipid were also present. The DNA G + C content of strain K110^T was 67.4 mol%. These chemotaxonomic properties supported the result of the monothetic phylogenetic classification, namely that strain K110^T could be a member of the genus *Pigmentiphaga*. Strain K110^T is phenotypically similar to *P. kullae*, as shown in Table 1. However, strain K110^T could be distinguished from *P. kullae* by notable differences in REP PCR genomic fingerprinting patterns (Supplementary Fig. S1 in IJSEM Online). DNA–DNA relatedness data were sufficient to categorize strain K110^T as representing a genomic species that is distinct from *P. kullae* (Wayne *et al.*, 1987). The mean value for DNA–DNA relatedness between strain K110^T and the type strain of *P. kullae* was 34.6% when their DNAs were used individually as labelled DNA probes for cross-hybridization, indicating that they represent different genomic species. Therefore, on the basis of the phenotypic, phylogenetic and genetic data, strain K110^T should be classified within the genus *Pigmentiphaga* as a member of a novel species, for which the name *Pigmentiphaga daeguensis* sp. nov. is proposed.

Description of *Pigmentiphaga daeguensis* sp. nov.

Pigmentiphaga daeguensis (dae.gu.en'sis. N.L. fem. adj. *daeguensis* of Daegu, Korea, from where the type strain was isolated).

Cells are Gram-negative, non-spore-forming rods (0.3–0.6 × 0.7–2.5 µm). Non-motile. Colonies on TSA are circular to slightly irregular, yellowish-white in colour and 1.0–2.0 mm in diameter after 3 days incubation at 37 °C. The optimal temperature for growth is 37 °C. Growth occurs at 15 and 46 °C, but not at 10 or 47 °C. The optimal pH for growth is 7.0–8.0; growth occurs at pH 5.0 and 9.0, but not at pH 4.5 or 9.5. Growth occurs in the presence of 0–5% (w/v) NaCl; optimal growth occurs in the presence of 0.5% (w/v) NaCl. No anaerobic growth occurs on TSA or on TSA supplemented with nitrate. Arginine dihydrolase activity is weakly positive. Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Adipate and malate are utilized, but glycerol, erythritol, D-arabinose, L-xylose, methyl β-D-xyloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, aesculin, lactose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, 2-ketogluconate, 5-ketogluconate, caprate and phenylacetate are not. The predominant ubiquinone is Q-8. The major fatty acids (>10% of total fatty acids) are C_{16:0}, cyclo C_{17:0} and cyclo C_{19:0ω8c}. The major polar lipids are phosphatidylglycerol, phosphatidylethanolamine and two unidentified aminolipids. The DNA G + C content is 67.4 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, K110^T (=KCTC 12838^T=JCM 14330^T), was isolated from wastewater from a dye works at Daegu, Korea.

Emended description of the genus *Pigmentiphaga* Blümel *et al.* 2001

The description of the genus *Pigmentiphaga* is as given by Blümel *et al.* (2001), with the following amendments. Cells are motile or non-motile. Common major polar lipid is phosphatidylethanolamine. The DNA G + C content is 67.4–68.8 mol%.

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