NOTE

***Pigmentiphaga kullae* gen. nov., sp. nov., a novel member of the family *Alcaligenaceae* with the ability to decolorize azo dyes aerobically**

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The taxonomic position of *Pseudomonas* strain K24, which was isolated previously after an aerobic enrichment with the azo compound 1-(4'-carboxyphenylazo)-4-naphthol as the sole source of carbon and energy, was investigated. The detection of a quinone system with ubiquinone Q-8 as the predominant compound and a polyamine pattern with putrescine and 2-hydroxyputrescine as the major polyamines present suggested that strain K24^T belongs to the β -subclass of the *Proteobacteria*. This was supported by sequencing the 16S rRNA gene, which demonstrated about 95–96% sequence similarity to different species of the genera *Achromobacter*, *Alcaligenes* and *Bordetella*. This suggested that strain K24^T is a member of the family *Alcaligenaceae*. The G+C content of the DNA was 68.5 mol%. Different methods for the construction of phylogenetic dendrograms placed strain K24^T separate from the genera *Alcaligenes*, *Achromobacter* and *Bordetella*. Analysis of the fatty acids demonstrated the presence of 10:0 3-OH and high concentrations of summed feature 7 (18:1 ω 7c, 18:1 ω 9t and/or 18:1 ω 12t) and 19:0 cyclo ω 8c, which is unique among previously described species of the genera *Alcaligenes*, *Achromobacter* and *Bordetella*. On the basis of the low 16S rRNA sequence similarities, the composition of the fatty acid profile and unique phenotypic properties, a new genus and species is proposed for strain K24^T with the name *Pigmentiphaga kullae* gen. nov., sp. nov.

Keywords: *Pigmentiphaga kullae*, degradation, azo compounds, *Alcaligenaceae*

One of the most impressive examples of the ability of micro-organisms to adapt to the utilization of xenobiotic compounds was a series of studies by Kulla and colleagues on the continuous adaptation of a mixed bacterial culture to the aerobic degradation of azo compounds (Kulla, 1981; Kulla *et al.*, 1983, 1984). In these studies, two different bacterial strains were isolated from the same inoculum that grew either with 1-(4'-carboxyphenylazo)-4-naphthol ('carboxy-Orange I') (strain K24^T) or 1-(4'-carboxyphenylazo)-2-naphthol ('carboxy-Orange II') (strain KF46). In the accompanying publication, a descendant of strain KF46 (strain KF46F^T) is identified as a new member

of the *Comamonadaceae*, *Xenophilus azovorans* (Blümel *et al.*, 2001). In the present manuscript, the taxonomic position of strain K24^T was analysed using the methods described in the accompanying manuscript (Blümel *et al.*, 2001). Strain K24^T was kindly provided by T. Leisinger (ETH Zürich, Switzerland). It has been deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) as DSM 13608^T and at the National Collection of Industrial and Marine Bacteria (Aberdeen, UK) as NCIMB 13708^T.

Strain K24^T formed visible colonies (diameter of about 1 mm) on agar plates with LB medium within 3 d at 30, 37 and 42 °C. No growth was found within 14 d at 4 °C. The colonies were opaque, circular and convex with an entire margin. The strain was oxidase- and

The GenBank accession number for the 16S rRNA gene sequence of strain K24^T is AF282916.

Fig. 1. Phylogenetic tree showing the relationship between *Pigmentiphaga kullae* K24^T and selected strains from other bacterial taxa. The tree was constructed using the maximum-parsimony method.

catalase-positive. Cells were motile, Gram-negative, non-spore-forming rods, about 2 µm in length.

The G + C content of the genomic DNA of strain K24^T was determined to be 68.5 ± 0.3 mol %.

The 16S rRNA sequence of strain K24^T, containing a continuous stretch of 1482 bp (positions 46–1502 according to the *Escherichia coli* numbering), was used to search the GenBank and Ribosomal Database Project libraries. Sequence searches showed that strain K24^T was most closely related phylogenetically to representatives of the β -subclass of the *Proteobacteria*. The results of the sequence similarity calculations indicated that the nearest relatives of strain K24^T are *Achromobacter ruhlandii* ATCC 15749^T, *Achromobacter xylosoxidans* subsp. *denitrificans* ATCC 15173^T and *Achromobacter xylosoxidans* subsp. *xylosoxidans* ATCC 27061^T (95.9–96.3 % sequence similarity). Only slightly lower sequence similarity values (95–95.8 %) were found to species from the genera *Alcaligenes* and *Bordetella*. Thus, it was evident that strain K24^T belongs to the family *Alcaligenaceae* as defined by De Ley *et al.* (1986). Furthermore, a significant degree of sequence similarity (94.0 %) was also observed to *Taylorella equigenitalis* NCTC 11184^T. Dendrograms were generated by using the maximum-parsimony, maximum-likelihood and neighbour-joining methods from the ARB program package (Ludwig & Strunk, 1997). The results of these trees grouped strain K24^T separately from the clades formed by the genera *Achromobacter*, *Alcaligenes* and *Bordetella* (Fig. 1). This was also confirmed by an analysis of signature oligonucleotides, which demonstrated a pattern that

was distinct from the three other genera within the *Alcaligenaceae* (Table 1).

Strain K24^T displayed a quinone system that consisted exclusively of ubiquinone Q-8. Analysis of the polyamines revealed a pattern for strain K24^T that is characteristic of taxa of the β -*Proteobacteria*, including members of the genera *Alcaligenes* and *Achromobacter* (Busse & Auling, 1988). Strain K24^T displayed the major compounds putrescine (55.4 µmol g⁻¹ dry weight) and 2-hydroxyputrescine (40.9 µmol g⁻¹ dry weight), whereas 1,3-diaminopropane, cadaverine, spermidine and spermine were detected only in minor amounts (0.03, 0.10, 0.22 and 0.02 µmol g⁻¹ dry weight, respectively).

Strain K24^T contained the fatty acids 16:0 (39.9 %), summed feature 3 (16:1 ISO I and 14:0 3-OH) (4.6 %), summed feature 7 (18:1 ω 7c, 18:1 ω 9t and/or 18:1 ω 12t) (9.8 %), 10:0 3-OH (2.9 %), 14:0 2-OH (4.0 %), 16:0 2-OH (4.8 %), 17:0 cyclo (21.9 %) and 19:0 cyclo ω 8c (12.2 %). A fatty acid profile similar to that of strain K24^T has not been described previously for any *Bordetella*, *Achromobacter* or *Alcaligenes* species (Foss *et al.*, 1998; Oyaizu-Masuchi & Komagata, 1988; Vandamme *et al.*, 1995, 1996). Among these taxa, the presence of 10:0 3-OH is unique and, together with the detection of high concentrations of summed feature 7 and 19:0 cyclo ω 8c, distinguishes strain K24^T clearly from any known species within this group.

The polar lipid profile of strain K24^T consisted of only three compounds. Phosphatidylethanolamine was detected as the major lipid and phosphatidylglycerol and

Table 1. Pattern of selected 16S rRNA signature nucleotides that define genera within the family *Alcaligenaceae*

Positions are given relative to the *E. coli* numbering. Nucleotides highlighted in bold indicate signature nucleotides that differentiate the respective genus from the other genera within the family. The 16S rRNA genes from the following organisms were analysed (accession numbers in parentheses): *Achromobacter piechaudii* ATCC 43552^T (AB010841), *Achromobacter ruhlandii* ATCC 15749^T (AB010840), *Achromobacter xylosoxidans* subsp. *denitrificans* ATCC 15173^T (M22509), *Achromobacter xylosoxidans* subsp. *xylosoxidans* ATCC 27061^T (D88005), *Alcaligenes defragrans* DSM 12141^T (AJ005447), *Alcaligenes faecalis* ATCC 8750^T (M22508), *Alcaligenes* sp. NKNTAU (U82826), *Bordetella avium* ATCC 35086^T (U04947), *Bordetella bronchiseptica* S-1 (X57026), *Bordetella holmesii* CDC F5101^T (U04820), *Bordetella parapertussis* ATCC 15311^T (U04949) and *Bordetella pertussis* ATCC 9797^T (U04950).

Position(s)	<i>Alcaligenes</i>	<i>Achromobacter</i>	<i>Bordetella</i>	K24 ^T
43:399	G*:C	G*:C	G:C	C:G
82:87	G:C	C:G	C:G	C:G
83	C	U	U	U
85	U	C	C	C
123:238	U:A	U:A	U:A	C:G
200:217	G:C	A:U	G:C	G:C
207:212	U:A	U:A	C:G	U:A
208	G	G	U	G
209	C	C	U	C
210	A	A	C	A
211	A	A	G	A
412	U	U	U	A
624:616	C:G	U:A	C†:G	C:G
673:717	A:U	G:C	G:C	G:C
1060:1197	U:A	U:A	U:A	C:G
1030	C	C	C	A
1137	C	A	A	A
1256	U	U	U	C
1283	U	C	C	C
1308	C	C	C	U
1329	G	G	G	A

* The nucleotides corresponding to position 43 were not available for the 16S rRNA genes of *Achromobacter ruhlandii*, *Achromobacter piechaudii* and *Alcaligenes* sp. NKNTAU.

† The sequence of *Bordetella avium* shows A at this position.

diphosphatidylglycerol were detected as minor spots after two-dimensional TLC.

Strain K24^T did not produce acids aerobically from various sugars and sugar alcohols tested. Of various *p*-nitrophenyl and *p*-nitroaniline derivatives, only L-alanine *p*-nitroaniline was hydrolysed. Only simple organic acids were utilized as sources of carbon and energy (for details, see species description below).

The family *Alcaligenaceae* consisted originally of the genera *Alcaligenes* and *Bordetella* (De Ley *et al.*, 1986).

Recently, the genus *Achromobacter* was revived to accommodate *Achromobacter xylosoxidans* subsp. *xylosoxidans*, *Achromobacter xylosoxidans* subsp. *denitrificans*, *Achromobacter ruhlandii* and *Achromobacter piechaudii*, assigned previously to the genus *Alcaligenes* (Yabuuchi *et al.*, 1998). Thus, the family *Alcaligenaceae* contains the three genera *Alcaligenes*, *Achromobacter* and *Bordetella*. Although not proposed so far, the genera *Taylorella* (Sugimoto *et al.*, 1983) and *Pelistega* (Vandamme *et al.*, 1998) may also be considered as members of the family due to their phylogenetic grouping close to *Alcaligenes faecalis*.

The 16S rRNA sequence, the presence of ubiquinone Q-8, putrescine and 2-hydroxyputrescine, the large amounts of 16:0 and 17:0 cyclo fatty acids and the G+C content of the DNA of strain K24^T were in accordance with the characteristics of the family *Alcaligenaceae*.

The three genera within the family *Alcaligenaceae* are currently difficult to differentiate on the basis of phenotypic features alone. Members of the genus *Bordetella* have to date been isolated almost exclusively from the respiratory tract or blood of animals, including humans (Vandamme *et al.*, 1996; Weiss, 1992). In contrast, members of the genera *Alcaligenes* and *Achromobacter* have been isolated mainly from the environment or as facultative pathogens from other locations in the human body. Unfortunately, there are no clear-cut physiological or chemotaxonomic differences known at present that separate the genera *Alcaligenes* and *Achromobacter*, and it was mainly analysis of 16S rDNA sequences that suggested that *Alcaligenes faecalis* ATCC 8750^T (the type species of the genus *Alcaligenes*) did not cluster together with the other previously known *Alcaligenes* strains and which give rise to the emendation of the genus *Achromobacter*. *Alcaligenes faecalis* can be distinguished clearly from *Achromobacter* species on the basis of a considerably lower G+C content (56 versus 65–69 mol %) (Yabuuchi *et al.*, 1998). However, the second species of the genus *Alcaligenes*, *Alcaligenes defragrans* DSM 12141^T, has a G+C content of about 67 mol % (Foss *et al.*, 1998). *Alcaligenes defragrans* DSM 12141^T clustered closely with *Alcaligenes* sp. NKNTAU, but more separate from *Alcaligenes faecalis* ATCC 8750^T, in all our phylogenetic trees (as shown in Fig. 1). Therefore, it can be assumed that *Alcaligenes defragrans* DSM 12141^T will be reclassified apart from the genus *Alcaligenes*.

Members of the genera *Achromobacter* and *Alcaligenes* may be distinguished on the basis of differences in their polar lipid profiles. *Achromobacter xylosoxidans* was described to contain diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as major lipids (Yabuuchi *et al.*, 1974). In contrast, *Alcaligenes faecalis* is reported to contain phosphatidylethanolamine, phosphatidylglycerol and an ornithine lipid and to lack diphosphatidylglycerol (Yabuuchi *et al.*, 1995). The detection of a polar lipid profile with the

single major component phosphatidylethanolamine and minor amounts of phosphatidylglycerol and diphosphatidylglycerol distinguishes strain K24^T from the genera *Alcaligenes* and *Achromobacter*.

The uniqueness of strain K24^T among the genera *Achromobacter*, *Alcaligenes* and *Bordetella* is evident from the sequence of the 16S rRNA gene, which showed the highest degrees of similarity of strain K24^T to *Achromobacter xylosoxidans* subsp. *denitrificans* ATCC 15173^T and *Achromobacter ruhlandii* ATCC 15749^T (96.3%). A closer affiliation of strain K24^T to the genus *Achromobacter* than to the other genera within the *Alcaligenaceae* is also suggested by the relatively high G+C content of the DNA and the polar lipid profile.

The 16S rRNA gene sequence similarity values among *Achromobacter* or *Bordetella* species are 97–98%. From this observation, it can be assumed that the threshold for differentiation of genera within the family *Alcaligenaceae* is <97% 16S rRNA gene sequence similarity. Since the 16S rRNA gene sequence similarity values between strain K24^T and species of *Achromobacter*, *Bordetella* and *Alcaligenes* were in the range 95–96.3%, it can be concluded that strain K24^T is a representative of an as yet undescribed genus. Furthermore, strain K24^T was clearly separated from all *Achromobacter* and *Alcaligenes* strains by the presence of 3-hydroxydecanoic acid (10:0 3-OH) and large amounts of the fatty acid 19:0 cyclo ω 8c.

Taking these results into consideration, we propose strain K24^T as representative of a new taxon, *Pigmentiphaga kullae* gen. nov., sp. nov.

Description of *Pigmentiphaga* gen. nov.

Pigmentiphaga (Pig.men'ti.pha.ga. L. n. *pigmentum* dye; Gr. n. *phagos* eater; N.L. fem. adj. *Pigmentiphaga* eating dyes).

Until further taxa are identified, the genus and species descriptions are combined below. The type species is *Pigmentiphaga kullae*.

Description of *Pigmentiphaga kullae* sp. nov.

Pigmentiphaga kullae (kul'lae. N.L. gen. n. *kullae* of Kulla, in honour of Hans G. Kulla, who initiated the work on the aerobic degradation of azo dyes by bacteria that resulted in the isolation of strain K24^T).

Gram-negative, motile, rod-shaped, non-spore-forming bacterium (1.3–4 μ m in length and 0.7–1.2 μ m in diameter). Colonies are opaque, circular and convex with an entire margin. The strain grows at 30, 37 and 42 °C; no growth at 4 °C. The strain is oxidase- and catalase-positive. L-Alanine *p*-nitroanilide is hydrolysed. The following compounds are not hydrolysed: *p*-nitrophenyl β -D-galactopyranoside, *p*-nitrophenyl β -D-glucuronide, *p*-nitrophenyl α -D-glucopyranoside, *p*-nitrophenyl β -D-glucopyranoside, *p*-nitrophenyl β -

D-xylopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl phenylphosphonate, bis-*p*-nitrophenyl phosphorylcholine, L-aniline *p*-nitroanilide, γ -L-glutamate *p*-nitroanilide and L-proline *p*-nitroanilide. The following compounds are used as sole sources of carbon: acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, mesaconate, 2-oxoglutarate, pyruvate, suberate, L-aspartate, 3-hydroxybenzoate and 4-hydroxybenzoate. The following compounds are not assimilated: *N*-acetyl-galactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-maltose, D-mannose, α -D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol, *i*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, 4-aminobutyrate, L-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. The G+C content of the DNA is 68.5 $\pm$ 0.3 mol%. The major isoprenoid quinone is ubiquinone Q-8. The main components in the polyamine pattern are putrescine and 2-hydroxyputrescine. The major fatty acids are 16:0, summed feature 3 (16:1 ISO I and 14:0 3-OH), summed feature 7 (18:1 ω 7c, 18:1 ω 9t and/or 18:1 ω 12t), 10:0 3-OH, 14:0 2-OH, 16:0 2-OH, 17:0 cyclo and 19:0 cyclo ω 8c. Phosphatidylethanolamine was detected as the major polar lipid and phosphatidylglycerol and diphosphatidylglycerol as minor components.

The type strain is strain K24^T (= DSM 13608^T = NCIMB 13708^T). Strain K24^T was isolated from soil after continuous enrichment with 1-(4'-carboxyphenylazo)-4-naphthol.

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