

## NOTE

***Pseudomonas kilonensis* sp. nov., a bacterium isolated from agricultural soil**Johannes Sikorski,<sup>1</sup> Erko Stackebrandt<sup>2</sup> and Wilfried Wackernagel<sup>1</sup><sup>1</sup> Genetik, Fachbereich Biologie, University of Oldenburg, D-26111 Oldenburg, Germany<sup>2</sup> Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

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**A total of 131 bacterial isolates related to *Pseudomonas corrugata* were obtained from an agricultural soil from northern Germany. Based on 16S rRNA gene sequences, PCR-based genome fingerprinting and multilocus enzyme electrophoresis, they formed two groups, A (119 strains) and B (12 strains). As members of each group were highly similar, a single strain of each group was subsequently characterized by a polyphasic taxonomic approach. The selected member of group A was identified as a strain of *Pseudomonas brassicacearum*, whereas the selected member of group B was distinct from other species of the genus *Pseudomonas*. Although DNA–DNA hybridization suggested a close affiliation of the group B strain with *P. brassicacearum* and *Pseudomonas thivervalensis* and ribotyping suggested a close affiliation with *P. brassicacearum*, RAPD data, 16S rRNA gene sequencing and phenotypic characterization indicated the presence of a distinct taxonomic entity. This strain differed from the type strains of *P. thivervalensis* and *P. brassicacearum* in 10 and 12 metabolic properties, respectively, whereas the two organisms differ from one another by only two properties. Strains of group B are therefore considered to be members of a new species, for which the name *Pseudomonas kilonensis* sp. nov. is proposed. The type strain is strain 520-20<sup>T</sup> (= DSM 13647<sup>T</sup> = CFBP 5372<sup>T</sup>).**

**Keywords:** *Pseudomonas kilonensis* sp. nov., DNA–DNA hybridization, RAPD-PCR, 16S gene nucleotide sequence, API, BIOLOG

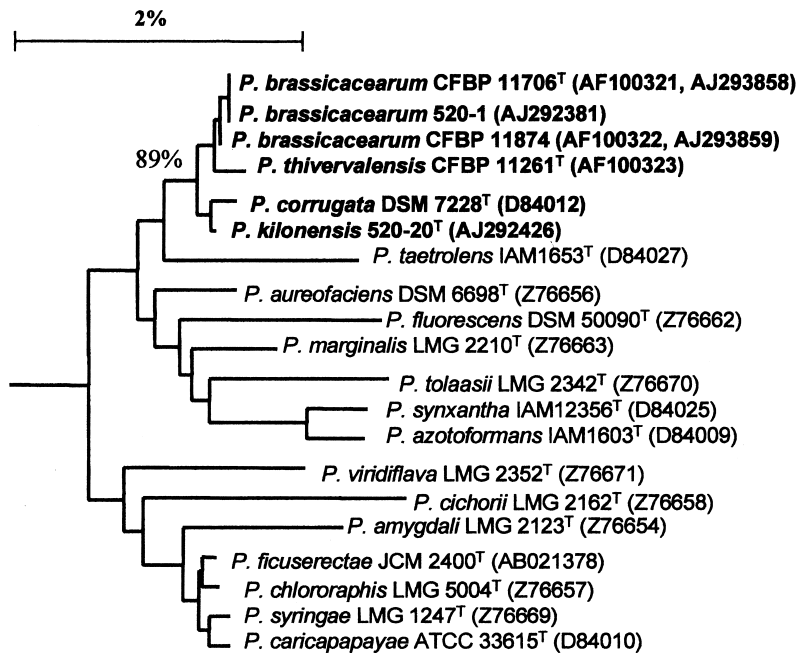
Since the original description of the genus *Pseudomonas* (Migula, 1894), members have been isolated from animals, including humans, and plants and from various environments, including marine and freshwater habitats, soil and sediment. Although the former phylogenetically polyphyletic genus *Pseudomonas* has been dissected (Kerstens *et al.*, 1996), even the authentic *Pseudomonas* species still show a wide distribution of environmental habitats and high phenotypic versatility (Moore *et al.*, 1996). We have recently isolated 131 strains from an agricultural soil near Kiel, Germany, that were related to the phytopathogenic species *Pseudomonas corrugata* on the basis of molecular typing. Characterization of the strains by PCR-based fingerprinting, multilocus enzyme electrophoresis and 16S rRNA gene sequencing indicated two coherent groups of strains termed A (119 strains) and B (12 strains) (Sikorski *et al.*, 2000). For studies on the

intraspecific diversity and population structure of these isolates, it was necessary to identify their affiliation with characterized *Pseudomonas* species by a polyphasic taxonomic approach, the results of which are presented below.

**Isolation of strains from soil**

A soil sample from the A<sub>p</sub> horizon of an agricultural field near Kiel (northern Germany) was suspended in sterile 0.9% NaCl and serial dilutions were plated on minimal medium (Lorenz & Wackernagel, 1991) with maltose as sole carbon source. After anaerobic incubation at 28 °C for 7 d, a total of 177 strains with a highly similar brownish gleaming colony type was isolated. The concentration of total bacterial counts on the initial plates was approx. 1 × 10<sup>5</sup> cells g<sup>-1</sup> soil and approx. 20% of them had the brownish gleaming colony type. After further purification and growth on LB medium, 131 of the 177 isolates maintained the highly similar colony morphology and colour. Characterization of the strains by PCR-based fingerprinting,

The GenBank/EMBL/DDBJ accession numbers for the *rrs* gene sequences of strains 520-1, 520-20<sup>T</sup>, CFBP 11076<sup>T</sup> and CFBP 11874 are respectively AJ292381, AJ292426, AJ293858 and AJ293859.



**Fig. 1.** Phylogenetic relationship of strains 520-1 and 520-20<sup>T</sup>. The phylogenetic tree is based on a neighbour-joining analysis of 33 16S rDNA sequences from 32 strains out of 30 *Pseudomonas* species and *E. coli* with 500 bootstrap replicates (Jukes & Cantor distances). Regions of 16S rDNA sequences that were available from all included strains (1396 nucleotide positions) were used. The tree is rooted with the *E. coli* sequence (accession no. J01695). Bar, 2% dissimilarity. *P. brassicacearum* strains CFBP 11706<sup>T</sup> and CFBP 11874 were positioned considering the corrected partial 16S rDNA sequences (Sikorski *et al.*, 2000), which have been deposited under accession numbers AJ293858 and AJ293859. These differ from the previously published sequences (AF100321 and AF100322; Achouak *et al.*, 2000) in four and two nucleotide positions, respectively.

multilocus enzyme electrophoresis and 16S rRNA gene sequencing indicated two coherent groups of strains termed A (119 strains) and B (12 strains) (Sikorski *et al.*, 2000).

### Molecular analysis

PCR amplification of 16S rRNA genes of strains 520-1 and 520-20<sup>T</sup> was carried out in 150 µl volumes containing approx. 12 ng genomic DNA, which was prepared with GeneReleaser (Eurogentec) as described by the manufacturer. The reaction mixtures contained 5% (v/v) DMSO, 1 µM each of universal primers fD1 and rD1 (Weisburg *et al.*, 1991), 50 µM of each dNTP (Pharmacia) and 1 U *Taq* DNA polymerase (Promega) in the reaction buffer supplied by the manufacturer. The cycling programme in a Perkin Elmer 480 thermocycler included an initial incubation at 92 °C for 5 min, 30 cycles of 92 °C for 2 min, 58 °C for 2 min and 70 °C for 3 min and a final extension at 70 °C for 10 min. The PCR product was sequenced using primers 5'-GTA-TTACCGCGGCTGCTGGC-3' and 5'-CAGCAGC-CGCGGTAATAC-3' (both positions 517–536 according to *Escherichia coli* numbering, in the forward and backward directions) and primer 5'-CTCCTAC-GGGAGGCAGCAG-3' (positions 339–357, *E. coli* numbering). Primer 5'-GGGTTGCGCTCGTTAC-GGG-3' (positions 1096–1114, *E. coli* numbering) was used to sequence a part (approx. positions 440–1080, *E. coli* numbering) of the PCR product from *Pseudomonas brassicacearum* strains CFBP 11706<sup>T</sup> and CFBP 11874. For phylogenetic analysis, the 16S rDNA sequences from 32 strains of 30 *Pseudomonas* species were included. The DNA sequences were aligned using CLUSTAL X 1.8 (Thompson *et al.*, 1997) and corrected manually. Phylogenetic analyses based upon 1396 nucleotides (ranging between *E. coli* positions 50 and

1445) were performed using a distance-based approach (neighbour-joining method with 500 bootstrap replicates with TREECON software; Van de Peer & De Wachter, 1994). Both strains were members of the *P. corrugata* lineage (Fig. 1). This cluster also contains the recently described and highly related *P. brassicacearum* (strains CFBP 11706<sup>T</sup> and CFBP 11874) and *Pseudomonas thivervalensis* (strain CFBP 11261<sup>T</sup>) (Achouak *et al.*, 2000). The binary similarity values of members of this lineage ranged between 99.2 and 99.8%. Strain 520-1 clustered with *P. brassicacearum* strains CFBP 11706<sup>T</sup> and CFBP 11874 and strain 520-20<sup>T</sup> clustered with *P. corrugata* CFBP 2431<sup>T</sup>. The separation of strain 520-20<sup>T</sup> from the *P. brassicacearum* strains and *P. thivervalensis* was supported both by a bootstrap value of 89% and by other phylogenetic analyses including maximum-parsimony and maximum-likelihood (Felsenstein, 1993).

### DNA–DNA hybridization

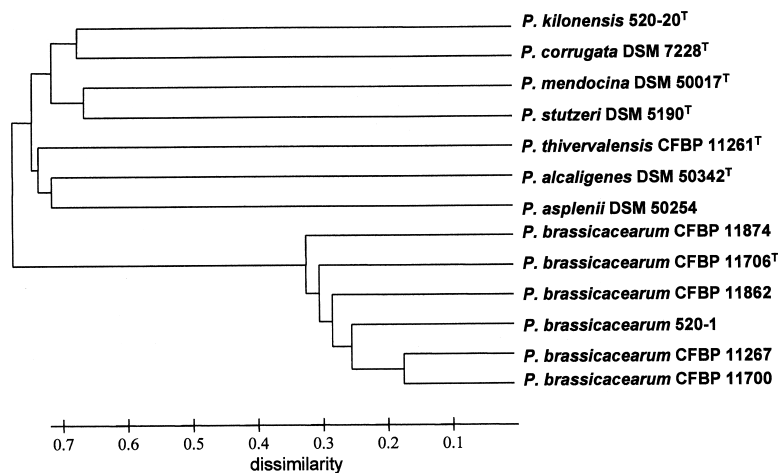
DNA was extracted and purified using a French press and hydroxyapatite chromatography as described previously (Cashion *et al.*, 1977). DNA–DNA hybridizations were carried out according to De Ley *et al.* (1970) using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter (Gilford Products Laboratories). The reassociation temperature was 70 °C. Renaturation rates and relatedness values were calculated as described previously (Jahnke, 1992). The high DNA relatedness value between strain 520-1 and *P. brassicacearum* CFBP 11706<sup>T</sup> (93%) suggests that it is a member of the latter species. Strain 520-20<sup>T</sup>, on the other hand, showed lower relatedness to the type strain of *P. brassicacearum* and to *P. brassicacearum* strain 520-1 (75 and 72%, respectively) and only moderate

**Table 1.** DNA–DNA hybridization values

Data in square brackets are from Achouak *et al.* (2000).

Strain	1	2	3	4
1. <i>P. kilonensis</i> 520-20 <sup>T</sup>	–	–	–	–
2. <i>P. brassicacearum</i> CFBP 11706 <sup>T</sup>	75	–	–	–
3. <i>P. brassicacearum</i> 520-1	72	93	–	–
4. <i>P. thivervalensis</i> CFBP 11261 <sup>T</sup>	69	61[61]	61	–
5. <i>P. corrugata</i> DSM 7228 <sup>T</sup>	51	[33]*	47	45[34, 46]

\* DNA–DNA hybridization was performed with *P. brassicacearum* CFBP 11699 instead of CFBP 11706<sup>T</sup> (Achouak *et al.*, 2000). The reassociation value between *P. brassicacearum* CFBP 11699 and CFBP 11706<sup>T</sup> was 96% (Achouak *et al.*, 2000).



**Fig. 2.** Genotypic characterization of strains 520-1 and 520-20<sup>T</sup> by RAPD-PCR. The dendrogram (UPGMA analysis, Nei & Li distances) is based on numerical analysis of 271 band positions from RAPD-PCR.

relatedness to the type strains of *P. thivervalensis* and *P. corrugata* (Table 1).

**DNA base composition**

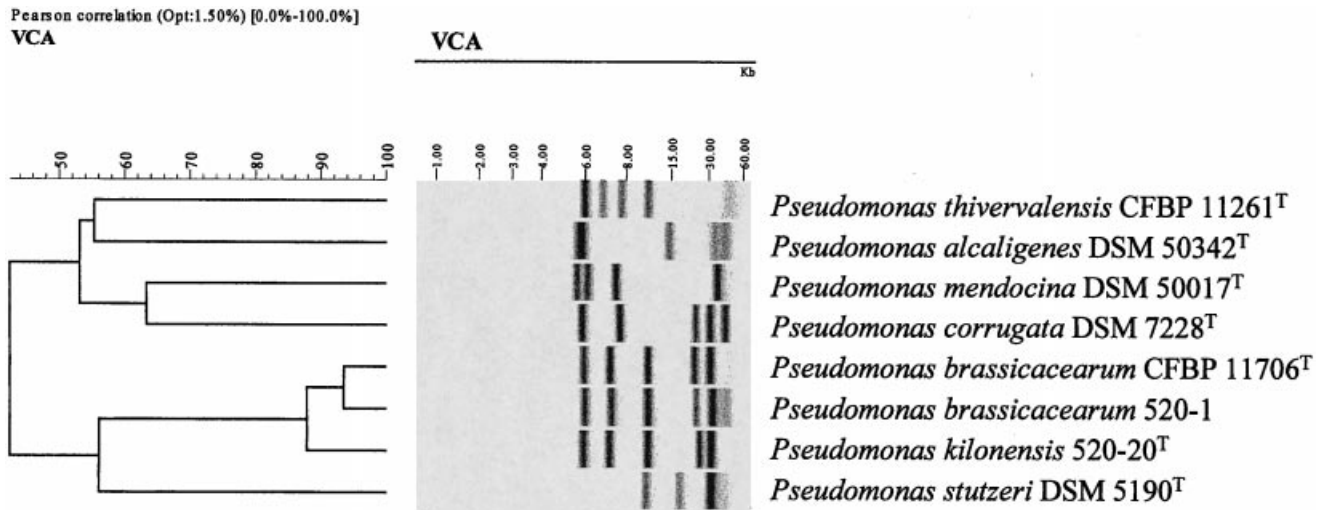
The G+C content of genomic DNA from strains 520-1 and 520-20<sup>T</sup> (both 59.9 mol%) was determined by HPLC as described previously (Mesbah *et al.*, 1989) using bacteriophage lambda DNA as the standard. These values are very similar to those of *P. brassicacearum* and *P. thivervalensis* (both 60.8 mol%; Achouak *et al.*, 2000) and *P. corrugata* (59.6 mol%; Sutra *et al.*, 1997).

**Genomic fingerprinting by RAPD-PCR**

A total of 131 strains, isolated independently on two occasions from a 250 g soil sample, were subjected to multilocus enzyme electrophoresis as a pre-sorting step and this indicated two distinct groups, A (119 strains) and B (12 strains). Additionally, 51 strains from group A and the 12 strains of group B were analysed by RAPD-PCR. The DNA was amplified in 25 µl reactions containing buffer (10 mM Tris/HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100 and 0.2 mg BSA ml<sup>-1</sup>), GeneReleaser DNA (1 µl),

one of the following six primers (0.2 µM), (1) 5'-CGAGCTTCGCGTACCACCCC-3', (2) 5'-GGAG-GTCACATGGAAGTCAG-3', (3) 5'-CTCAATGG-CAGCGGCTATGG-3', (4) 5'-GTTTCGCTCGAT-GCGCTACC-3', (5) 5'-CCCTGGCCGAAATCAC-CCCC-3' or (6) 5'-CTACGGCAACGCGACGCT-GAC-3', 1 U *Taq* DNA polymerase (Promega) and 100 µM of each dNTP (Pharmacia) under a drop of mineral oil (Sigma). A Perkin-Elmer TC 480 thermal cycler was used for amplification: four cycles at 94 °C, 40 °C and 70 °C for 5 min each followed by 30 cycles at 94 °C and 55 °C for 1 min each and 70 °C for 2 min, with a final primer extension at 70 °C for 5 min. PCR products were resolved on agarose gels (1.3%). A size marker (Ladder Mix, MBI Fermentas) was used as a reference in all gels. The RAPD patterns were analysed with the software GENE IMAGIR version 3.52 (Scanalytics) and exported into the TRECON software (Van de Peer & De Wachter, 1994) to generate a UPGMA dendrogram from a distance matrix (Nei & Li, 1979).

RAPD-PCR supported the two clearly distinct groups, A and B. One strain of each group [*P. brassicacearum* strain 520-1 (group A) and strain 520-20<sup>T</sup> (group B)], together with 11 reference strains, are discussed here (Fig. 2). The RAPD-PCR with six primers yielded a



**Fig. 3.** Riboprint patterns of *Eco*RI-restricted DNA of some type strains of *Pseudomonas* species and soil isolates. The analysis was done with the RiboPrinter Microbial Characterization System (Qualicon). The riboprint pattern was analysed with the BioNumerics software package GENEXPLORÉ version 1.0 (Applied Maths). VCA is the RiboPrinter internal designation for an *Eco*RI batch.

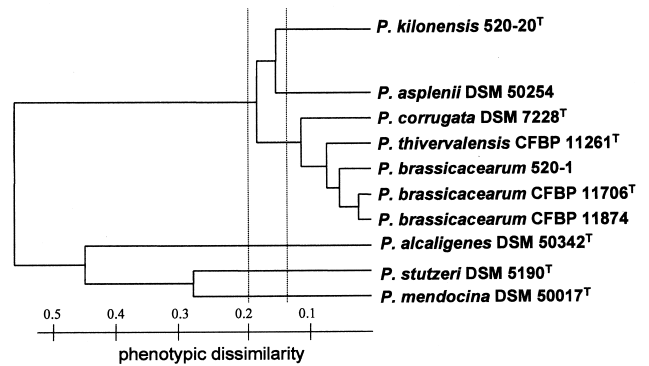
total of 271 band positions. The strains of *P. brassicacearum* (including strain 520-1) showed some molecular heterogeneity and clustered at a distance of 0.32. The patterns of members of group B (including strain 520-20<sup>T</sup>) were almost identical. The various type strains and strain 520-20<sup>T</sup> differed from each other at a distance of 0.66–0.78.

**Ribotyping**

Strain 520-20<sup>T</sup>, *P. brassicacearum* 520-1 and the type strains of *P. thivervalensis*, *Pseudomonas alcaligenes*, *Pseudomonas mendocina*, *P. corrugata*, *P. brassicacearum* and *Pseudomonas stutzeri* were ribotyped with the restriction enzyme *Eco*RI. All strains possessed at least three bands, ranging in size between 6.0 and 50 kb. As displayed in the dendrogram of relatedness (Fig. 3), the type strains of *P. corrugata* and *P. thivervalensis* branched separately from the type strain of *P. brassicacearum*, supporting the results of the DNA–DNA reassociation experiments (Table 1). The close relatedness of the riboprint patterns of *P. brassicacearum* 520-1 and the type strain of *P. brassicacearum* is in agreement with the physiological and molecular data. The patterns of these two strains were rather similar to the pattern of strain 520-20<sup>T</sup>, which is in agreement with the results of the DNA–DNA reassociation study.

**Metabolic profiles**

Enzymic activities, oxidation of carbons substrates and assimilation of carbon substrates were determined using API20 NE and API50 CH strips (bioMérieux) and BIOLOG GN MicroPlates as recommended by



**Fig. 4.** Phenotypic dendrogram (UPGMA analysis, Nei & Li distances) based on numerical analysis of the results of API20 NE and API50 CH tests.

the manufacturers. BIOLOG results were read with a BIOLOG MicroStation System (MicroLog GN 3.50 database) after 24 and 48 h incubation at 28 °C. API strips were read visually after 24 and 48 h incubation at 28 °C. For strain characterization, only values taken after 48 h were taken into account.

The dendrogram of the phenotypic distances between the 10 strains is shown in Fig. 4 and the data are included in Table 2. *P. alcaligenes*, *P. stutzeri* and *P. mendocina* clearly clustered apart from the other strains, which are grouped together at a distance of 0.2. The remaining strains could be subdivided at a distance of 0.14 into three phenons containing strain 520-20<sup>T</sup> (phenon 1), *Pseudomonas asplenii* (phenon 2) and the strains of *P. corrugata*, *P. thivervalensis* and *P. brassicacearum* (phenon 3). Phenon 1 can be differ-

**Table 2.** Biochemical characters that differentiate between strains of *P. kilonensis*, *P. brassicacearum*, *P. thivervalensis*, *P. corrugata* and *P. asplenii*

Strains are identified as: 1, *P. kilonensis* 520-20<sup>T</sup>; 2–7, *P. brassicacearum* strains 520-1 (2), CFBP 11706<sup>T</sup> (3), CFBP 11874 (4), CFBP 11862 (5), CFBP 11700 (6) and CFBP 11267 (7); 8, *P. thivervalensis* CFBP 11261<sup>T</sup>; 9, *P. corrugata* DSM 7228<sup>T</sup>; 10, *P. asplenii* DSM 50254. Characters are scored as: +, positive; –, negative; D, variable; NT, not tested. Data in square brackets were taken from Achouak *et al.* (2000).

Character*	1	2	3	4	5	6	7	8	9	10
Nitrate reduction <sup>a</sup>	–	–	–	+	–	–	–	–	+	–
Arginine dihydrolase <sup>a</sup>	–	+	+	+	+	+	+	+	–	+
Gelatin hydrolysis <sup>a</sup>	+	+	+	+	+	+	+	+	+	–
Assimilation of <i>N</i> -acetylglucosamine <sup>a</sup>	–	+	+	+	+	+	+	+	+	–
Oxidation of:										
Glycerol <sup>b</sup>	–	–	–	–	NT	NT	NT	+	+	+
Ribose <sup>b</sup>	+	–	–	–	NT	NT	NT	–	+	+
Inositol <sup>b</sup>	–	–	+	+	NT	NT	NT	+	+	–
Mannitol <sup>b</sup>	–	+	+	+	NT	NT	NT	+	+	–
Sorbitol <sup>b</sup>	–	+	+	+	NT	NT	NT	+	–	–
Melibiose <sup>b</sup>	+	–	–	–	NT	NT	NT	–	–	–
Sucrose <sup>b</sup>	+	+	+	+	NT	NT	NT	+	+	–
Trehalose <sup>b</sup>	+	+	+	+	NT	NT	NT	+	+	–
D-Fucose <sup>b</sup>	+	+	–	–	NT	NT	NT	+	+	+
D-Galacturonic acid <sup>c</sup>	–	+	[+]†	[+]	[+]	[+]	[+]	[D]†	NT	NT
D-Glucuronic acid <sup>c</sup>	–	+	[+]†	[+]	[+]	[+]	[+]	[D]†	NT	NT
Sebacic acid <sup>c</sup>	–	–	[+]†	[+]	[+]	[+]	[+]	[+]†	NT	NT
Succinamic acid <sup>c</sup>	–	+	[+]†	[+]	[+]	[+]	[+]	[–]†	NT	NT
L-Pyroglutamic acid <sup>c</sup>	–	+	[+]†	[+]	[+]	[+]	[+]	[+]†	NT	NT
L-Ornithine <sup>c</sup>	–	+	[+]†	[+]	[+]	[+]	[+]	[D]†	NT	NT
Putrescine <sup>c</sup>	+	–	[–]†	[–]	[–]	[–]	[–]	[D]†	NT	NT

\* Characters were determined with API20 NE (a), API 50 CH (b) or BIOLOG GN (c) substrates.

† Data from Achouak *et al.* (2000) representing six strains, including the type strain.

entiated from phenons 2 and 3 by several biochemical characters (Table 2).

### Taxonomic conclusions

The aim of this study was to characterize undescribed soil isolates that belong to the *P. corrugata* subline of the authentic pseudomonads. Two representatives of the emerging clusters were selected (strains 520-1 and 520-20<sup>T</sup>). On the basis of DNA–DNA hybridization, 16S rRNA gene sequence analysis, ribotyping, RAPD-PCR and analysis of metabolic properties, strain 520-1 was determined to be a member of *P. brassicacearum*, whereas strain 520-20<sup>T</sup> could not be affiliated unambiguously with any existing taxonomic group at the species level. Based solely upon DNA relatedness, strain 520-20<sup>T</sup> should be considered a strain of *P. brassicacearum*. The issue of the importance of such borderline value in a discipline in which taxonomy is increasingly performed in the framework of molecular and phylogenetic characterization of strains has already been raised by Vandamme *et al.* (1996). Moreover, the threshold value, which is described as a recommendation only (Wayne *et al.*, 1987), is often used in a very inflexible way in the literature. Also, the

DNA hybridization similarity between strain 520-20<sup>T</sup> and *P. thivervalensis* almost reached the 70% border value. The two species *P. brassicacearum* and *P. thivervalensis* can be distinguished phenotypically and by ribotyping but are closely related phylogenetically (16S rRNA similarity). Despite a slightly higher similarity at the 16S rRNA level, strain 520-20<sup>T</sup> is only moderately related to *P. corrugata* at the level of overall DNA similarity (51% DNA relatedness to the type strain). This is supported by the ribotyping data, which indicated a weaker affiliation of strain 520-20<sup>T</sup> with *P. corrugata* and *P. thivervalensis* than with *P. brassicacearum* (Fig. 3). The conclusion that strain 520-20<sup>T</sup> and other members of group B constitute a taxonomically independent entity worth of species status originates from RAPD-PCR data and the analysis of metabolic properties. Genomic fingerprinting techniques have previously demonstrated their suitability for clustering strains according to their overall genomic relatedness (Rademaker *et al.*, 2000; Sikorski *et al.*, 1999). Based upon BIOLOG analyses, strain 520-20<sup>T</sup> differed from *P. brassicacearum* in six properties (oxidation of D-galacturonic acid, D-glucuronic acid, succinamic acid, L-pyroglutamic acid, L-ornithine and putrescine) and from *P. thivervalensis* in

two (oxidation of sebacic acid and L-pyroglutamic acid). In addition, the API analysis revealed six differences from the strains of *P. brassicacearum* (arginine dihydrolase activity, assimilation of *N*-acetylglucosamine and oxidation of ribose, mannitol, sorbitol and melibiose) and eight differences from *P. thivervalensis* (arginine dihydrolase activity, assimilation of *N*-acetylglucosamine and oxidation of glycerol, ribose, inositol, mannitol, sorbitol and melibiose). Variable traits of strains of *P. brassicacearum* in API analyses (nitrate reduction and oxidation of inositol and D-fucose) and BIOLOG analyses (oxidation of sebacic acid) and of *P. thivervalensis* in BIOLOG analyses (oxidation of D-galacturonic acid, D-glucuronic acid, L-ornithine and putrescine; Achouak *et al.*, 2000) were not taken into account. Thus, there are significantly more metabolic differences between strain 520-20<sup>T</sup> and the strains of *P. brassicacearum* and *P. thivervalensis* than between the latter two type strains.

On the basis of this evidence, we propose the name *Pseudomonas kilonensis* sp. nov. for strain 520-20<sup>T</sup> and 11 additional strains that share the same RAPD-PCR pattern, with strain 520-20<sup>T</sup> as the type strain.

#### Description of *Pseudomonas kilonensis* sp. nov.

*Pseudomonas kilonensis* (ki.lo.nen'sis. L. adj. *kilonensis* of Kiel, after *Civium Kilonensis*, the ancient Latin name of the town of Kiel, Germany).

Cells from stationary culture of strain 520-20<sup>T</sup> are motile rods, 1.0–2.0 µm in length and about 0.5 µm in diameter. The white-yellowish colonies on King B medium are smooth with regular margins and, after 2–3 d, produce a yellow diffusible pigment giving a light yellow-green fluorescence when irradiated with UV light (254 nm). After 2–3 weeks, colonies on King B medium produce a dark brown pigment that colours the colony and the agar. Results obtained with API20 NE strips indicate that strains have the following enzyme activities: hydrolysis (protease) and oxidase. All other enzymic activities included in the API20 NE strips are negative. Results obtained with API20 NE strips indicate that strains utilize the following substrates as carbon and energy sources: arabinose, caprate, citrate, gluconate, glucose, malate, mannitol and mannose. All other substrates included in the API20 NE strips were not utilized. Results obtained with API50 CH strips indicate that strains oxidize the following substrates: L-arabinose, D-fructose, D-fucose, galactose, D-glucose, D-mannose, melibiose, ribose, sucrose, trehalose and D-xylose. Results obtained with BIOLOG GN microplates indicate that strains oxidize the following substrates: acetic acid, *N*-acetyl D-glucosamine, alaninamide, D-alanine, L-alanine, L-alanyl-glycine,  $\gamma$ -aminobutyric acid, 2-aminoethanol, L-arabinose, D-arabitol, L-asparagine, L-aspartic acid, *cis*-aconitic acid, bromosuccinic acid, DL-carnitine, citric acid, formic acid, D-fructose, D-galactonic acid lactone, D-galactose,  $\alpha$ -D-glucose,

D-gluconic acid, D-glucosaminic acid, L-glutamic acid, glycerol, glycyl L-glutamic acid, L-histidine,  $\beta$ -hydroxybutyric acid,  $\gamma$ -hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, hydroxy-L-proline, inosine, *m*-inositol,  $\alpha$ -ketoglutaric acid, DL-lactic acid, L-leucine, malonic acid, D-mannitol, D-mannose, methylpyruvate, monomethylsuccinate, L-proline, propionic acid, putrescine, quinic acid, D-saccharic acid, L-serine, D-sorbitol, succinic acid, sucrose, D-trehalose, L-threonine, Tween 40, Tween 80, uridine and urocanic acid. Oxidation of the other organic substrates included in BIOLOG GN microplates is negative.

The type strain is strain 520-20<sup>T</sup> (= DSM 13647<sup>T</sup> = CFBP 5372<sup>T</sup>), isolated from agricultural soil near Kiel, Germany.

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