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Hexachlorocyclohexane-degrading bacterial strains *Sphingomonas paucimobilis* B90A, UT26 and Sp+, having similar *lin* genes, represent three distinct species, *Sphingobium indicum* sp. nov., *Sphingobium japonicum* sp. nov. and *Sphingobium francense* sp. nov., and reclassification of [*Sphingomonas*] *chungbukensis* as *Sphingobium chungbukense* comb. nov.

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Three strains of *Sphingomonas paucimobilis*, B90A, UT26 and Sp+, isolated from different geographical locations, were found to degrade hexachlorocyclohexane. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these strains do not fall in a clade that includes the type strain, *Sphingomonas paucimobilis* ATCC 29837^T, but form a coherent cluster with [*Sphingomonas*] *chungbukensis* IMSNU 11152^T followed by *Sphingobium chlorophenolicum* ATCC 33790^T. The three strains showed low DNA–DNA relatedness values with *Sphingomonas paucimobilis* ATCC 29837^T (8–25 %), [*Sphingomonas*] *chungbukensis* IMSNU 11152^T (10–17 %), *Sphingobium chlorophenolicum* ATCC 33790^T (23–54 %) and *Sphingomonas xenophaga* DSM 6383^T (10–28 %), indicating that they do not belong to any of these species. Although the three strains were found to be closely related to each other based on 16S rRNA gene sequence similarity (99·1–99·4 %), DNA–DNA relatedness (19–59 %) and pulsed-field gel electrophoresis (PFGE) patterns indicated that they possibly represent three novel species of the genus *Sphingobium*. The three strains could also be readily distinguished by biochemical tests. The three strains showed similar polar lipid profiles and contained sphingoglycolipids. The strains differed from each other in fatty acid composition but contained the predominant fatty acids characteristic of other *Sphingobium* species. A phylogenetic study based on 16S rRNA gene sequences showed that [*Sphingomonas*] *chungbukensis* IMSNU 11152^T formed a cluster with members of the genus *Sphingobium*. Based on these results, it is proposed that strains B90A, UT26 and Sp+, previously known as *Sphingomonas paucimobilis*, are the type strains of *Sphingobium indicum* sp. nov. (=MTCC 6364^T=CCM 7286^T), *Sphingobium japonicum* sp. nov. (=MTCC 6362^T=CCM 7287^T) and *Sphingobium francense* sp. nov. (=MTCC 6363^T=CCM 7288^T), respectively. It is also proposed that [*Sphingomonas*] *chungbukensis* be transferred to *Sphingobium chungbukense* comb. nov.

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Abbreviation: HCH, hexachlorocyclohexane.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Sphingobium indicum* sp. nov. MTCC 6364^T and *Sphingobium francense* sp. nov. MTCC 6363^T are AY519129 and AY519130, respectively.

A table showing DNA–DNA relatedness values for strains B90A, UT26, Sp+ and *Sphingomonas paucimobilis* ATCC 29837^T is available as supplementary material in IJSEM Online.

Introduction

Hexachlorocyclohexane (HCH) has been used extensively for the control of agricultural pests and in health programmes all over the world for more than 50 years. Two forms of HCH have been used: lindane (gamma-HCH) and technical HCH (a mixture of four HCH isomers; alpha-, beta-, gamma- and delta-HCH). Extensive and prolonged use of gamma-HCH and technical-HCH has resulted in the evolution of aerobic bacterial strains capable of degrading HCH isomers (Dogra *et al.*, 2004). Three such strains of *Sphingomonas paucimobilis*, SS86 (UT26), Sp+ and B90A, have been isolated from different geographical locations (Senoo & Wada, 1989; Sahu *et al.*, 1990; Dogra *et al.*, 2004). The mutant strain UT26 is resistant to nalidixic acid and is derived from the original SS86 strain that was isolated in 1989 from upland fields in Japan where gamma-HCH had been applied for 15 years (Senoo & Wada, 1989; Wada *et al.*, 1989). Strain Sp+ was isolated from French soils contaminated with gamma-HCH presumably for only a few years (Dogra *et al.*, 2004). Both UT26 and Sp+ were found to degrade alpha-, gamma- and delta-HCH, but neither strain was able to degrade beta-HCH. Strain B90A, isolated in 1989 from rice rhizosphere soil in India under repeated treatment with technical HCH (Sahu *et al.*, 1990), degraded all HCH isomers, including beta-HCH, but at different rates (Sahu *et al.*, 1990; Johri *et al.*, 1998; Kumari *et al.*, 2002; Dogra *et al.*, 2004). The catabolic genes responsible for the degradation of HCH isomers found in the three strains are almost identical (Nagata *et al.*, 1999; Kumari *et al.*, 2002; Dogra *et al.*, 2004). These genes, *linA*, *linB*, *linC* (*linX*), *linD* and *linE*, encode HCH dehydrochlorinase, halidohydrolase, dehydrogenase, dechlorinase and ring cleavage dioxygenase, respectively. Interestingly, B90A was found to contain multiple copies of *linA* and *linX*, compared with only one copy of each gene in Sp+ and UT26 (Kumari *et al.*, 2002; Dogra *et al.*, 2004). All *lin* genes in these strains are associated with IS*6100* insertion sequences (Dogra *et al.*, 2004). This prompted us to determine the taxonomic position of these three strains. A polyphasic investigation was conducted using 16S rRNA gene sequences, DNA–DNA hybridization, fatty acid and polar lipid analyses and biochemical parameters. The data obtained show that each of these three strains constitutes a different species of *Sphingobium* and that they do not represent any currently known species. The novel species are characterized and described. While classifying the three novel strains, we noticed that [*Sphingomonas*] *chungbukensis* IMSNU 11152^T also clustered with species of the recently described genus *Sphingobium*. Thus, we also propose to transfer [*Sphingomonas*] *chungbukensis* to *Sphingobium chungbukense* comb. nov.

16S rRNA gene sequencing and analysis

Total DNA of strains B90A, UT26 and Sp+ was isolated according to Kumari *et al.* (2002). PCR amplification of 16S rRNA genes and DNA sequencing were carried out as described by Reddy *et al.* (2000). The CLUSTAL_X program (Thompson *et al.*, 1997) from the European Bioinformatics Institute website (http://www.ebi.ac.uk/) was used to align the sequences. The 16S rRNA gene sequences of the three strains, B90A, UT26 and Sp+, showed similarity values of 91·9–92·7 % when compared with that of the type strain of *S. paucimobilis*, ATCC 29837^T. This indicated that they are probably not members of the species *Sphingomonas paucimobilis*. Between the three strains, 16S rRNA gene sequence similarity was 99·1–99·4 %. Similarity searches showed that the 16S rRNA gene sequences of B90A, UT26 and Sp+ were most similar to those of [*Sphingomonas*] *chungbukensis* IMSNU 11152^T (98·5–98·8 %) and *Sphingobium chlorophenolicum* ATCC 33790^T (98·1–98·4 %).

The nearly full-length 16S rRNA gene sequences of the 25 species with validly published names of the genera *Sphingomonas* and *Sphingobium*, as well as the sequence of *Rhodanobacter lindaniclasticus* RP 5557^T (Nalin *et al.*, 1999), which also contains *lin* genes, were retrieved from GenBank. These sequences and those of the three study strains were aligned using CLUSTAL_X. The alignment was checked manually for quality and the terminal nucleotides not common to all 29 sequences were removed. Phylogenetic analysis was carried out using the PHYLIP package, version 3.5 (Felsenstein, 1993). The evolutionary distance matrix was calculated using the distance model of Jukes & Cantor (1969). The evolutionary tree (Fig. 1) was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the resultant tree topologies were evaluated by bootstrap analysis based on 100 resamplings, using the SEQBOOT and CONSENSE programs in the PHYLIP package (Fig. 1). Strains B90A, UT26 and Sp+ were in a monophyletic clade represented by species of the genus *Sphingobium* as well as [*Sphingomonas*] *chungbukensis* IMSNU 11152^T. Strains B90A, UT26 and Sp+ showed 16S rRNA gene sequence similarity to [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and *Sphingobium chlorophenolicum* ATCC 33790^T ranging from 98·5 to 98·8 % and 98·1 to 98·4 %, respectively. 16S rRNA gene sequence similarity between [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and species of *Sphingobium* in the monophyletic cluster ranged between 95·6 and 98·1 % (Kim *et al.*, 2000), with a similarity of 98·1 % between [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and *Sphingobium chlorophenolicum* ATCC 33790^T. Parsimony analysis was also performed for the aligned sequence data using DNAPARS including bootstrap analysis with 100 resamplings. This gave a tree topology very similar to that obtained with the distance method (data not shown).

DNA–DNA hybridization

16S rRNA gene sequence similarity and the phylogenetic analysis showed that B90A, UT26 and Sp+ are closely related to each other. They were found to be clustered in a coherent group with [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and *Sphingobium chlorophenolicum* ATCC 33790^T. In order to clarify their taxonomic status, DNA–DNA hybridization tests were carried out for these two type

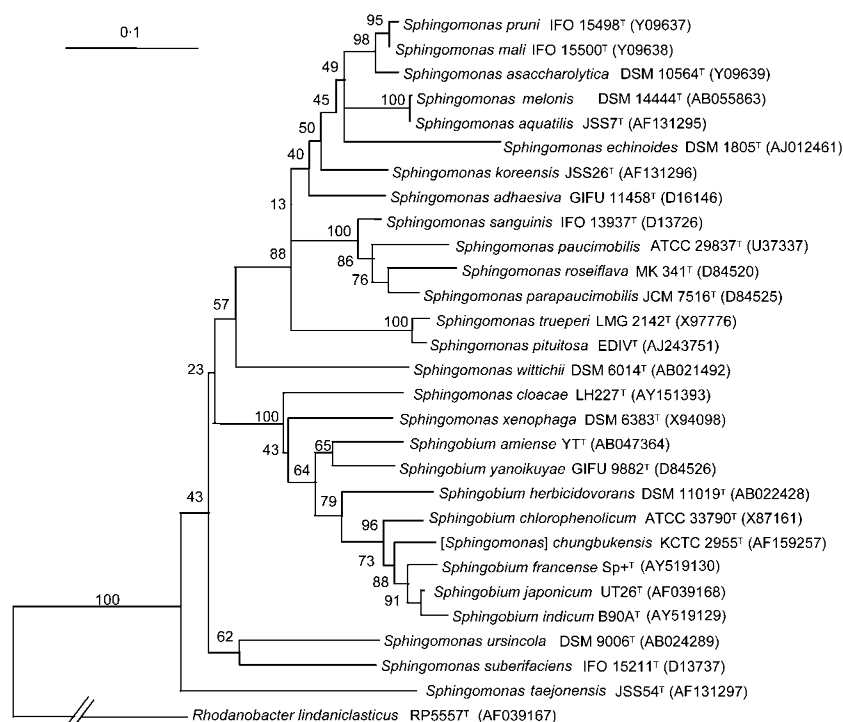

Fig. 1. Phylogenetic tree based on almost-complete 16S rRNA gene sequences (1444 aligned positions) showing the relationship between strains B90A, UT26 and Sp+. The tree was constructed by the neighbour-joining method and rooted by using *Rhodanobacter lindaniclasticus* as the out-group. Numbers at nodes represent boot-strap values (based on 100 resamplings). The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. Twenty five species with validly published names were used to construct the phylogenetic tree. Bar, 0·1 nucleotide substitution per nucleotide position.

strains and for *Sphingomonas paucimobilis* ATCC 29837^T and *Sphingomonas xenophaga* DSM 6383^T, a strain that also degrades polycyclic aromatic hydrocarbons. DNA–DNA hybridization was carried out by the membrane filter method (Tourova & Antonov, 1987). DNA–DNA relatedness between the strains was determined according to Bala *et al.* (2004). DNA (10 μg) of each strain was transferred onto a positively charged nylon membrane (Hybond-N; Amersham) using a dot blot apparatus (Bio-Rad). The membrane was air-dried and cross-linked. The DNA probe for each strain was labelled with [α-P^{32}]-ATP (BRIT) using a nick-translation kit (Amersham Pharmacia). Hybridization was performed overnight at 65 °C. After hybridization, the filter was washed with SSC and SDS to remove unbound probe. The amount of the probe bound to the DNA was estimated using a scintillation counter (Beckman Instruments) and hybridization values obtained were expressed as percentage of the probe bound relative to the homologous reaction. DNA from each of the seven strains was bound to the filters and hybridized with DNA probes prepared from each strain. The DNA–DNA hybridization data (Supplementary Table S1 in IJSEM Online) showed that B90A, UT26 and Sp+ had DNA–DNA relatedness values of 13, 10 and 8 % respectively with the type strain *Sphingomonas paucimobilis* ATCC 29837^T. The DNA–DNA relatedness values between B90A, UT26 and Sp+ varied from 19 to 59 %. [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and *Sphingobium chlorophenolicum* ATCC 33790^T were not only more closely related to B90A, UT26 and Sp+ based on 16S rRNA gene sequence (Fig. 1), but the genomic DNA–DNA relatedness values also indicated that these strains are more closely related to [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and *Sphingobium chlorophenolicum* ATCC 33790^T than to *Sphingomonas paucimobilis* ATCC 29837^T (see Supplementary Table S1 in IJSEM Online). All DNA–DNA hybridization values were below the threshold value (70 %) that has been suggested as delineating a bacterial species (Wayne *et al.*, 1987). These data support the results of 16S rRNA gene sequence analysis and lend support to the view that B90A, UT26 and Sp+ represent three distinct species of *Sphingobium*.

Primarily based on the phylogenetic analysis of 16S rRNA gene sequences of the then known species of *Sphingomonas* and some chemotaxonomic and phenotypic differences among them, Takeuchi *et al.* (2001) proposed the creation of three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, in addition to the genus *Sphingomonas sensu stricto*. The genus *Sphingobium* was proposed to accommodate a group of species that formed a monophyletic cluster, including *Sphingobium chlorophenolicum*, *Sphingobium herbicidivorans* and *Sphingobium yanoikuyae*. However, the 16S rRNA gene sequence and description of [*Sphingomonas*] *chungbukensis* IMSNU 11152^T (Kim *et al.* 2000) were not available at the time that Takeuchi *et al.* (2001) made their proposal for the new genus *Sphingobium*. Since [*Sphingomonas*] *chungbukensis* IMSNU 11152^T falls within a monophyletic cluster represented exclusively by species of the genus *Sphingobium*, it also appears to belong to the genus *Sphingobium* (Fig. 1).

DNA profiling, IS*6100* and evolution of *lin* genes

Six strains of the genera *Sphingomonas* and *Sphingobium*, B90A, UT26, Sp+, *Sphingomonas paucimobilis* ATCC

29837^T, *Sphingobium chlorophenolicum* ATCC 33790^T and *Sphingomonas xenophaga* DSM 6383^T, were examined by pulsed-field gel electrophoresis (PFGE). DNA was prepared in agarose blocks using the protocol recommended by Bio-Rad. Restriction digestion was performed according to Pandza *et al.* (1997). Electrophoresis was conducted in 0·5 × TBE buffer at 14 °C using a CHEF DR III system (Bio-Rad). Lambda DNA concatemers (Amersham) were used as molecular mass standards. Digests were performed with the rarely cutting restriction enzymes *Xba*I and *Ase*I. *Xba*I produced ranges of fragment sizes which could be resolved well by PFGE. *Xba*I digests of the six strains were distinctly different (data not shown) with no discernible resemblance between them (data not shown). Although the results of PFGE profiling alone cannot be used to describe the strains as three different species of *Sphingobium*, they provide additional support to the results of 16S rRNA gene sequencing and DNA–DNA hybridization.

While there are differences between the strains as regards 16S rRNA gene sequences, DNA–DNA relatedness and PFGE profiling, each strain possesses highly similar *lin* genes, which are associated with copies of IS*6100* insertion sequences (Kumari *et al.*, 2002; Dogra *et al.*, 2004). Strains B90A, UT26 and Sp+ contain 11, 5 and 6 copies of IS*6100*, respectively, at different chromosomal positions. We therefore decided to investigate the presence of IS*6100* and *lin* genes in *Sphingomonas paucimobilis* ATCC 29837^T, [*Sphingomonas*] *chungbukensis* IMSNU 11152^T, *Sphingobium chlorophenolicum* ATCC 33790^T and *Sphingomonas xenophaga* DSM 6383^T by Southern blot hybridization and PCR amplification. None of these strains carried *linA*, *linB*, *linC* (*linX*), *linD* or *linE* (data not shown). Interestingly, the *linA* genes of B90A, UT26 and Sp+, while nearly identical, showed a strange mosaic configuration (Dogra *et al.*, 2004). It has been suggested that *linA* was transferred into the common ancestor by IS*6100*, followed by geographical dispersion (Kumari *et al.*, 2002; Dogra *et al.*, 2004). However, the present work shows that the three strains differ from each other so much that they should be considered to represent separate species. This makes it more likely that the *lin* genes have been transferred into the three strains as a result of independent events from an, as-yet unknown, host strain.

Polar lipid and fatty acid analyses

Polar lipid and cellular fatty acid analyses of B90A, UT26 and Sp+ were carried out by the DSMZ by the following procedures. Polar lipids were analysed according to the method of Tindall (1990a, b). Fatty acid methyl esters were analysed from 40 mg cells scraped from a Petri dish and subjected to saponification, methylation and extraction using the method of Miller (1982) and Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated using the Sherlock Microbial Identification System (Microbial ID) which consisted of a gas chromatograph (model 5980; Agilent) fitted with a 5 % phenyl-methyl silicone column (0·2 mm × 25 m) and FID detector. Identification and comparison were made using the Aerobe (TSBA50, version 5) database of the Sherlock Microbial Identification System. As shown in Table 1, the polar lipid profiles of B90A, UT26 and Sp+ were almost identical. While phosphatidylmonomethylethanolamine (PMME) and phosphatidylethanolamine (PE) were abundant in all three strains, diphosphatidylglycerol (DPG) was a major

Table 1. Polar lipid distribution in sphingomonads

Strains: 1, *Sphingomonas paucimobilis* B90A (data from this study); 2, *Sphingomonas paucimobilis* UT26 (this study); 3, *Sphingomonas paucimobilis* Sp+ (this study); 4, *Sphingomonas paucimobilis* DSM 1098^T (Busse *et al.*, 1999); 5, *Sphingomonas xenophaga* DSM 6383^T (Busse *et al.*, 1999); 6, *Sphingobium chlorophenolicum* ATCC 33790^T (Busse *et al.*, 1999). ++, Present in major amount; +, minor amount; −, not detected.

Polar lipids	1	2	3	4	5	6
Phosphatidylmonomethylethanolamine (PMME)	++	++	++	+	+	+
Phosphatidylethanolamine (PE)	++	++	++	++	++	++
Phosphatidylglycerol (PG)	+	+	+	++	++	++
Diphosphatidylglycerol (DPG)	++	+	+	+	++	++
Phosphatidylcholine (PC)	+	+	+	++	+	+
Sphingoglycolipid (SGL)*	++	++	++	++	++	++
SGL2/unidentified glycolipid (GL2)†	+	+	+	+	−	−
Phosphatidyldimethylethanolamine (PDE)	−	−	−	+	++	++
Unidentified phospholipids (PL):						
PL-3	−	−	−	+	+	+
PL-4	−	−	−	−	++	+
Unidentified lipid (L-1)	−	−	−	−	++	−

*SGL represents SGL1.

†SGL2 represents GL2 of *Sphingomonas paucimobilis* DSM 1098^T (Busse *et al.*, 1999).

polar lipid in B90A, but not in Sp+ or UT26. The major sphingoglycolipid SGL1 was present in all the strains and SGL2 was present in small amounts. SGL1 and SGL2 correspond to SGL and GL2, respectively, of *Sphingomonas paucimobilis* DSM 1098^T (Busse *et al.*, 1999). Several polar lipids (PDE and PL3) reported as present in *Sphingomonas paucimobilis* DSM 1098^T (Busse *et al.*, 1999), *Sphingomonas xenophaga* DSM 6383^T (Busse *et al.*, 1999) and *Sphingobium chlorophenolicum* ATCC 33790^T (Busse *et al.*, 1999) were absent in B90A, UT26 and Sp+. With respect to fatty acid profiles, B90A, UT26 and Sp+ again showed minor qualitative and quantitative differences. Fatty acid 18:1ω7*c* predominated, with relatively high levels of 16:0 and a major 2-hydroxy fatty acid 14:0 (Table 2), features common to most of the *Sphingobium* species (Takeuchi *et al.*, 2001). Additionally, a similarity index of all the isolates produced from the Aerobe TSBA50 library showed that the three strains fell within the group represented by the genus *Sphingobium*. The cellular fatty acid compositions of B90A, UT26 and Sp+ were similar to those of other *Sphingobium* species (Takeuchi *et al.*, 2001). Qualitative and quantitative differences in fatty acid composition between the three strains and larger qualitative and quantitative differences when they were compared with *Sphingomonas paucimobilis* ATCC 29837^T further suggest that the three strains possibly represent three distinct species of the genus *Sphingobium*.

The cellular fatty acid composition of [*Sphingomonas*] *chungbukensis* IMSNU 11152^T was not only closer to B90A, UT26 and Sp+ (this study), but also closer to those of *Sphingobium chlorophenolicum* DSM 7098^T, *Sphingobium herbicidovorans* DSM 11019^T and *Sphingobium yanoikuyae* KCTC 2818^T (Kim *et al.*, 2000), further distinguishing [*Sphingomonas*] *chungbukensis* IMSNU 11152^T from the genus *Sphingomonas*.

Table 2. Cellular fatty acid profiles of sphingomonad strains used in this study

Values are percentages of total fatty acid content (w/w). Strains: 1, *Sphingomonas paucimobilis* B90A (data from this study); 2, *Sphingomonas paucimobilis* UT26 (this study); 3, *Sphingomonas paucimobilis* Sp+ (this study); 4, *Sphingomonas paucimobilis* DSM 1098^T (Busse *et al.*, 1999); 5, [*Sphingomonas*] *chungbukensis* IMSNU 11152^T (Kim *et al.*, 2000); 6, *Sphingomonas xenophaga* DSM 6383^T (Busse *et al.*, 1999); 7, *Sphingobium chlorophenolicum* ATCC 33790^T (Busse *et al.*, 1999). −, Not detected.

Fatty acid	1	2	3	4	5	6	7
14:0	−	0·17	0·16	1·4	−	−	−
14:0 2-OH	6·84	4·30	5·06	6·4	9·0	6·7	9·4
Summed feature:*							
3	8·41	13·78	8·23	−	−	−	−
4	−	−	−	2·7	6·0	23·9	9·6
7	−	−	−	74·6	67·0	55·2	60·1
15:0 2-OH	0·20	−	−	−	−	−	−
16:0	18·96	10·04	16·46	8·7	12·0	8·0	9·5
16:0 2-OH	0·24	−	0·23	−	−	−	−
16:1ω5*c*	0·92	2·18	1·19	−	1·0	2·0	2·4
17:1ω6*c*	−	−	1·88	3·0	2·0	2·5	6·4
18:0	0·89	−	0·36	−	−	−	−
18:1ω7*c*	57·66	66·70	62·50	−	−	−	−
18:1ω5*c*	1·64	1·40	1·10	3·2	3·0	1·6	2·5
11-methyl 18:1ω7*c*	4·25	1·29	2·84	−	−	−	−

*Summed features consist of one or more fatty acids that could not be separated. Summed feature 3 and 4 contained one or more of the following fatty acids: 16:1ω*c* and 15:0 iso 2-OH. Summed feature 7 contained one or more of the following isomers: 18:1ω7*c*, 18:1ω9*t* and/or 18:1ω12*t*.

Phenotypic properties

Liquid cultures of the three strains were grown in mineral salts medium (SM) supplemented with 1% glucose (SMG) as described previously (Kumari *et al.*, 2002). The morphological characteristics (colour, shape, size and contour of colonies) of B90A, UT26 and Sp+ were studied on nutrient agar, SM agar medium and LB agar plates. Growth at different temperatures and catalase tests were carried out as described by McCarthy & Cross (1984). All three strains were non-motile, Gram-negative rods (Table 3). The cell size of B90A, UT26 and Sp+ varied from 1·0 to 2·5 μm. The optimum temperature for growth was 28 °C. UT26 and Sp+ produced yellow colonies. Colonies of strain B90A were initially yellow, with later production of a dark-brown soluble pigment which diffused into the LB agar plates. The dark-brown pigment was analysed by spectrophotometry and the peak absorbance was found at 532·0 nm. The production of brownish water-soluble pigment appears to be an interesting feature and has been reported earlier for *Sphingobium herbicidovorans* DSM 11019^T (Zipper *et al.*, 1996), which is located at the *Sphingobium chlorophenolicum* ATCC 33790^T branch.

All the strains were subjected to a battery of physiological tests. Hydrolysis of Tween 20 and Tween 80 and the ability of the strains to grow in the presence of NaCl were tested as described by Arden-Jones *et al.* (1979). Acid production from carbohydrates and degradation of xanthine and hypoxanthine were tested according to Gordon *et al.* (1974). Urease activity was detected as in Christensen (1946). The other physiological tests and methods were as described by Collins *et al.* (1989). To determine antibiotic sensitivity, the bacterial strains were grown in SM broth supplemented with 100 μg ampicillin ml^{-1}, 25 μg chloramphenicol ml^{-1}, 50 μg kanamycin ml^{-1}, 15 μg tetracycline ml^{-1} and 10 μg erythromycin ml^{-1}. A comparison was made (Table 3), wherever possible, with previously described test results for [*Sphingomonas*] *chungbukensis* IMSNU 11152^T (Kim *et al.*, 2000), *Sphingobium chlorophenolicum* ATCC 33790^T (Nohynek *et al.*, 1996) and *Sphingomonas xenophaga* DSM 6383^T (Stolz *et al.*, 2000). The degradation of HCH was studied as described

Table 3. Physiological characteristics of sphingomonad strains

Strains: 1, *Sphingomonas paucimobilis* B90A; 2, *Sphingomonas paucimobilis* UT26; 3, *Sphingomonas paucimobilis* Sp+; 4, [*Sphingomonas*] *chungbukensis* (data from Kim *et al.*, 2000); 5, *Sphingobium chlorophenolicum* (Nohynek *et al.*, 1996); 6, *Sphingomonas xenophaga* (Stolz *et al.*, 2000). Strains 1–3 all assimilated glucose, galactose, arabinose and xylose but lactose, rhamnose, inositol, erythritol, adonitol, mannitol, sorbitol, raffinose and mannose were not assimilated; none of them degraded xanthine, hypoxanthine, casein or gelatin and no H_2S or citrate production was observed. Strains 1–3 were sensitive to even 5 % NaCl and were oxidase-positive. All strains formed circular colonies. −, Negative; W, weakly positive; +, positive; ND, not determined.

Characteristic	1	2	3	4	5	6
Colour	Yellow	Yellow	Yellow	Yellow	Bright yellow	Yellow
Colony size (mm)	0·2–1·0	0·2–1·0	0·2–1·0	0·2–2·0	0·2–3·0	0·2–1·0
Degradation of HCH:						
Alpha	+	+	+	−	−	−
Beta	+	−	+	−	−	−
Delta	+	+	+	−	−	−
Gamma	+	+	+	−	−	−
Production of soluble pigment on:						
Nutrient agar medium	+	−	−	−	−	−
LB medium	+	−	−	+	+	+
Acid production from trehalose	+	W	−	ND	ND	ND
Assimilation of:						
Cellobiose	−	+	+	−	+	+
Fructose	+	+	−	+	−	−
Ribose	+	W	W	ND	−	−
Sucrose	−	+	−	ND	−	+
Degradation of:						
Aesculin	−	+	+	+	+	+
Tween 20*	1+	2+	1+	ND	ND	ND
Tween 80	−	+	−	ND	ND	ND
Production of catalase*	1+	2+	3+	ND	ND	ND
Sensitive to:						
Ampicillin (100 μg ml^{-1})	−	−	−	ND	ND	ND
Chloramphenicol (25 μg ml^{-1})	+	+	+	ND	ND	ND
Erythromycin (10 μg ml^{-1})	+	−	+	ND	ND	ND
Kanamycin (50 μg ml^{-1})	+	+	+	ND	ND	ND
Tetracycline (15 μg ml^{-1})	+	+	+	ND	ND	ND

*1+, Low activity; 2+, moderate activity; 3+, strong activity.

previously (Kumari *et al.*, 2002; Dogra *et al.*, 2004). Table 3 shows that B90A, UT26 and Sp+ can be differentiated from each other on the basis of several tests. The phenotypic features of B90A, UT26 and Sp+, were more common to [*Sphingomonas*] *chungbukensis* IMSNU 11152^T and *Sphingobium chlorophenolicum* ATCC 33790^T (Table 3); the study strains, including [*Sphingomonas*] *chungbukensis* IMSNU 11152^T, differed in several features from *Sphingomonas* species (data not shown)

Based on the results described above, we conclude that the three HCH-degrading strains of *Sphingomonas paucimobilis* represent three novel species of the genus *Sphingobium*. The names *Sphingobium indicum* sp. nov., *Sphingobium japonicum* sp. nov. and *Sphingobium francense* sp. nov. are proposed for strains B90A, UT26 and Sp+, respectively. We also propose to transfer [*Sphingomonas*] *chungbukensis* to *Sphingobium chungbukense* comb. nov.

Description of *Sphingobium indicum* sp. nov.

Sphingobium indicum (in.di′cum. L. neut. adj. *indicum* referring to India, from where the type strain was isolated).

Gram-negative, rod-shaped, aerobic, mesophilic, non-spore-forming, non-motile bacterium. Colonies (0·2–1·0 mm in diameter) are yellow, smooth and circular. Slimy growth and produces water-soluble brown pigment on LB as well as on nutrient medium. It degrades all the isomers (alpha-, beta-, gamma- and delta-) of HCH. The degradation of alpha- and gamma-HCH is nearly 100 % (complete mineralization), but incomplete degradation of delta- and beta-HCH. Resistant

to ampicillin (100 μg ml^{-1}). Catalase-, urease- and oxidase-positive. Acid is produced from glucose, arabinose, trehalose and galactose. No acid production from sucrose, lactose, rhamnose, inositol or adonitol. The following sugars are assimilated: glucose, galactose, trehalose, arabinose, ribose, xylose and fructose. The following sugars are not assimilated: sucrose, lactose, rhamnose, erythritol, inositol, adonitol, cellobiose, mannitol, sorbitol, mannose and raffinose. No degradation of aesculin, Tween 80, xanthine, hypoxanthine or casein. No production of H_2S, citrate or methyl red. Sensitive to 5 % NaCl. Contains two and three copies of the *linA* and *linX* genes, respectively. Also contains 11 copies of IS*6100* and one copy of each of the *linB*, *linC*, *linD* and *linE* and *linR* genes. The major polar lipids are phosphatidylmonomethylethanolamine, phosphatidylethanolamine and diphosphatidylglycerol. Major fatty acids are 18 : 1ω7*c*, saturated 16 : 0 and 2-hydroxy fatty acid 14 : 0 (Table 2).

The type strain, B90A^T (=MTCC 6364^T=CCM 7286^T), was isolated from the rice rhizosphere, Cuttack, India.

Description of *Sphingobium japonicum* sp. nov.

Sphingobium japonicum (ja.po.ni′cum. N.L. neut. adj. *japonicum* referring to Japan, from where the type strain was isolated).

Gram-negative, rod-shaped, aerobic, mesophilic, non-spore-forming, non-motile bacterium. Colonies (0·2–1·0 mm in diameter) are yellow, smooth and circular. Slimy growth and does not produce pigment on LB or on nutrient medium. Utilizes alpha-, gamma- and delta-HCH as a source of carbon, but not beta-HCH. Resistant to ampicillin (100 μg ml^{-1}), erythromycin (10 μg ml^{-1}) and nalidixic acid. Catalase- and oxidase-positive. Acid is produced from glucose, arabinose, trehalose and galactose. The following compounds are assimilated: fructose, sucrose, galactose, cellobiose and ribose. Aesculin, Tween 20 and Tween 80 are hydrolysed. Contains one copy of each of the *linA*, *linB*, *linC*, *linD*, *linR* genes and five copies of IS*6100*. The major polar lipids are phosphatidylmonomethylethanolamine (PMME) and phosphatidylethanolamine (PE). Major fatty acids are 18 : 1ω7*c*, saturated 16 : 0 and 2-hydroxy fatty acid 14 : 0.

The type strain, UT26^T (=MTCC 6362^T=CCM 7287^T), was isolated from upland soils in Japan that had been treated repeatedly with gamma-HCH for 15 years.

Description of *Sphingobium francense* sp. nov.

Sphingobium francense (fran.cen′se. N.L. neut. adj. *francense* referring to France, from where the type strain was isolated).

Gram-negative, rod-shaped, aerobic, mesophilic, non-spore-forming, non-motile bacterium. Degrades all isomers of HCH. Colonies (0·2–1·0 mm in diameter) are yellow, smooth and circular. Resistant to ampicillin (100 μg ml^{-1}). Catalase- and oxidase-positive. Acid is produced from glucose, arabinose, galactose, cellobiose and ribose. Assimilates cellobiose and ribose. Aesculin and Tween 20 are hydrolysed. The major polar lipids are phosphatidylmonomethylethanolamine and phosphatidylethanolamine. Major fatty acids are 18 : 1ω7*c*, saturated 16 : 0 and 2-hydroxy fatty acid 14 : 0 (Table 2). Contains one copy of each of the *linA*, *linB*, *linD* and *linE* genes, two copies of *linC* and six copies of IS*6100*.

The type strain, Sp+^{T} (=MTCC 6363^T=CCM 7288^T), was isolated from gamma-HCH-contaminated soils in France.

Description of *Sphingobium chungbukense* (Kim *et al.* 2000) comb. nov.

Sphingobium chungbukense (chung.bu.ken′se. N.L. neut. adj. *chungbukense* named after Chungbuk National University).

Basonym: *Sphingomonas chungbukensis* Kim *et al.* 2000.

The description is identical to the description given for *Sphingomonas chungbukensis* by Kim *et al.* (2000). The type strain is DJ77^T (=KCTC 2955^T=IMSNU 11152^T=JCM 11454^T).

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