**Sphingobium baderi** sp. nov., isolated from a hexachlorocyclohexane dump site

Jasvinder Kaur,1† Hana Moskalikova,2† Neha Niharika,1† Miroslava Sedlackova,3 Ales Hampl,2,3 Jiri Damborsky,2,4 Zbynek Prokop4 and Rup Lal1

1Molecular Biology Laboratory, Department of Zoology, University of Delhi, Delhi-110007, India
2International Clinical Research Centre, St Anne’s University Hospital Brno, Pekarska 53, 656 91 Brno, Czech Republic
3Department of Histology and Embryology, Faculty of Medicine, Masaryk University, 625 00 Brno, Czech Republic
4Loschmidt Laboratories and Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, 628 00 Brno, Czech Republic

A Gram-stain-negative, rod-shaped and white-coloured bacterial strain, designated LL03T, was isolated from hexachlorocyclohexane-contaminated soil at Spolana Neratovice, Czech Republic, where lindane was formerly produced. Strain LL03T was found to be a degrader of \( \alpha-, \gamma- \) and \( \delta- \) isomers of hexachlorocyclohexane, although no significant degradation activity was observed for the \( \beta- \) isomer. A neighbour-joining tree based on 16S rRNA gene sequences showed that strain LL03T occupied a distinct phylogenetic position in the *Sphingobium* cluster, showing the highest similarity with *Sphingobium wenxiniae* JZ-1T (99.2 %). The DNA G+C content of strain LL03T was 67.0 mol%. DNA–DNA relatedness values of strain LL03T with its close phylogenetic neighbours were below the threshold level of 70 %, supporting its identification as a representative of a novel species of the genus *Sphingobium*. The predominant respiratory quinone was ubiquinone Q-10. The polar lipid profile of strain LL03T also corresponded to those reported for other *Sphingobium* species (phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and sphingoglycolipid), supporting its identification as a member of the genus *Sphingobium*. Spermidine was identified as the major polyamine. The predominant fatty acids were 16:0, summed feature 3 (16:1ω7c and/or 16:1ω6c), summed feature 8 (18:1ω7c and/or 18:1ω6c) and 14:0 2-OH. The polar lipid pattern, the presence of spermidine and ubiquinone Q-10, the predominance of the cellular fatty acids C18:1ω7c, C16:0 and C14:0 2-OH and the G+C content of the genomic DNA supported the affiliation of the strain to the genus *Sphingobium*. The results obtained after DNA–DNA hybridization, biochemical and physiological tests clearly distinguished it from closely related species of the genus *Sphingobium*. Therefore, strain LL03T represents a novel species of the genus *Sphingobium* for which the name *Sphingobium baderi* LL03T sp. nov. is proposed; the type strain is LL03T (=CCM 7981T =DSM 25433T).

**Abbreviations:** HCH, hexachlorocyclohexane; t-HCH, technical hexachlorocyclohexane.

†These authors contributed equally to this work.

The GenBank accession number for 16S rRNA gene sequence of strain LL03T (=CCM 7981T =DSM 25433T) is JN695620.

Two supplementary figures and a supplementary table are available with the online version of this paper.

Dedication: The authors dedicate this article to Dr Alfred Bader.

Hexachlorocyclohexane (HCH) is a saturated chlorinated hydrocarbon and consists of eight variously stable isomers. Out of these isomers, \( \gamma- \)HCH or lindane, was widely used from the 1940s to the 1990s as a pesticide. Since lindane production is a highly inefficient process wherein 90 % of the production mix is waste, HCH emerged as a major environmental pollutant. The toxicity assessment of HCH isomers ranked them as probable human carcinogens, endocrine disruptors with proven teratogenic, genotoxic, and mutagenic effects (ATSDR, 2005). In an attempt to selectively isolate strains capable of degrading HCH, soil
samples were taken from the ground below the former production facility of Spolana Neratovice, Czech Republic. Strain LL03\(^T\) was isolated by the enrichment method of Ito and co-workers (Ito et al., 2007). Strain LL03\(^T\) was found to degrade \(\alpha\)-, \(\gamma\)- and \(\delta\)- isomers, while no significant degradation activity was observed for the \(\beta\)-isomer of HCH. It is interesting to note that the strain is novel in the respect that it lacks the \(\text{linB}\) gene which encodes the enzyme involved in the degradation of \(\beta\)-HCH. None of the neighbouring strains could degrade HCH except for Sphingobium quisquiliarum P25\(^T\), which could also degrade \(\alpha\)- and \(\gamma\)-HCH completely but degraded \(\delta\)-HCH only slightly following 24 h incubation. Preliminary studies with the strain showed it to be a member of the genus Sphingobium. The genus Sphingobium, grouped in the family Sphingomonadaceae, belongs to the Alphaproteobacteria. The genus was established together with Novosphingobium and Sphingopyxis by Takeuchi et al. (2001). It forms cluster II in the phylogenetic tree of Sphingomonas species (Takeuchi et al., 2001). Members of the family are widely distributed in nature and are known to degrade a variety of compounds (Lal et al., 2008). At the time of writing, the genus Sphingobium consisted of 25 species. Here, we analysed the taxonomic status of strain LL03\(^T\) by using a polyphasic approach (Prakash et al., 2007a).

A soil sample (1 g) was mixed with 2 ml 1/10 W medium (1:1; KH\(_2\)PO\(_4\), 170 mg; Na\(_2\)HPO\(_4\), 980 mg; (NH\(_4\))\(_2\)SO\(_4\), 100 mg; MgSO\(_4\), 48.7 mg; FeSO\(_4\), 0.52 mg; MgO, 10.75 mg; CaCO\(_3\), 2.0 g; ZnSO\(_4\), 0.81 mg; CuSO\(_4\), 0.16 mg; CoSO\(_4\), 0.15 mg; and H\(_3\)BO\(_3\), 0.06 mg) and vortexed. The mixture was centrifuged (1000 \times g, 30 min) and 1 ml supernatant was then inoculated into 100 ml 1/10 W medium saturated with technical HCH (t-HCH). After static incubation at 25 °C for 10 days, 1 ml of the primary enrichment culture was transferred into 100 ml of fresh 1/10 W medium, and the resultant secondary enrichment culture was incubated under the same condition for 4 days. The procedure of transfer and incubation was once repeated. Serial dilutions of the tertiary enriched culture were spread on 1/10 W agar plates containing 1.8 mM t-HCH. After incubation at 25 °C for 15 days, a number of colonies with a cleared zone of degraded t-HCH were picked up. Single-colony isolation was repeated several times on t-HCH minimal medium to obtain pure cultures. Strain LL03\(^T\) was found to be a member of the genus Sphingobium for which the name Sphingobium baderi LL03\(^T\) is proposed for it.

16S rRNA gene sequence analysis was carried out as described by Prakash et al. (2007b). A 1462 bp stretch of 16S rRNA gene sequence was obtained, which was checked manually to ensure quality of sequences. Searches for closely related species were carried out using the BLAST program (Altschul et al., 1997) from the NCBI (http://www.ncbi.nlm.nih.gov). The nearly full-length 16S rRNA gene sequences of species closely related to LL03\(^T\) were retrieved from GenBank for the construction of phylogenetic trees. The 16S rRNA gene sequence of Zymomonas mobilis ATCC 10988\(^T\) was used as an outgroup. All the selected sequences were aligned using the CLUSTAL_X program, version 1.81b (Thompson et al., 1997). The alignment was checked manually for quality. The evolutionary distance matrix was calculated using the distance model of Jukes & Cantor (1969) within the TREECON software package, version 1.3b (Van de Peer & De Wachter, 1994). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) and the resultant tree topology was evaluated by bootstrap analysis based on 1000 resamplings. The tree topology was found to be similar with maximum-parsimony, neighbour-joining and maximum-likelihood methods. Evaluation of the topology revealed that strain LL03\(^T\) clustered with members of the genus Sphingobium. The signature nucleotides found in the 16S rRNA gene sequence characteristic for the genus Sphingobium sensu stricto (Takeuchi et al., 2001) were found in the 16S rRNA gene sequence of strain LL03\(^T\). These nucleotides were U: A at position 52: 359, G at position 134, A: U at position 987: 1218 and U: G at position 990: 1215, based on Escherichia coli numbering (Brosius et al., 1978). This observation further supported the placement of strain LL03\(^T\) within the genus Sphingobium.

Strain LL03\(^T\) showed the highest 16S rRNA gene sequence similarity (99.2 %) with Sphingobium wenxiniae JZ-1\(^T\) (Wang et al., 2011; Fig. 1). DNA–DNA hybridization was carried out between strain LL03\(^T\) and closely related strains showing more than 97 % sequence similarity of 16S rRNA gene sequence with LL03\(^T\). Total genomic DNA of all the closely related strains was extracted, purified and hybridization was done by following the protocol as described by Kumar et al. (2008) and Tourova & Antonov (1988). 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, cross-linked and the DNA probe for each strain was labelled with [\(\alpha\)-\(^32\)P]-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 probe bound to the DNA was estimated using a \(\beta\)-scintillation counter (Perkin Elmer) and hybridization values obtained were expressed as percentage of the probe bound relative to the homologous reaction. All the DNA–DNA hybridization values were below the threshold value of 70 % (Table S1, available in IJSEM Online), as is recommended for the delineation of bacterial species (Wayne et al., 1987), thus confirming that strain LL03\(^T\) represents a novel species of the genus Sphingobium. Cell morphology was examined using electron microscopy (Fig. S1). Samples were fixed in 300 mmol glutaraldehyde 11\(^{-1}\) in 100 mmol cacodylate buffer 1\(^{-1}\) and post-fixed in 40 mmol osmium tetroxide 1\(^{-1}\) in 100 mmol cacodylate buffer 1\(^{-1}\). For negative staining, the samples were spread on EM grids (coated with Formvar carbon support film), incubated for 15 s in 2 % solution of ammonium molybdate; then the excess solution was removed, samples were air-dried at...
were 16:0 (14.3%), summed feature 3 (16:1t)
identification System (MIDI). The predominant fatty acids
version 60) database of the Sherlock Microbial Iden-
The mixture of FAMEs was separated by GC (Agilent 6890)
ultramicro-
sections were cut using Leica EM UC6 ultramicro-
sections were examined under an FEI Morgagni 268D
tome and stained with uranyl acetate and lead citrate.
(60 nm thick) were cut using Leica EM UC6 ultramicro-
protocol described by Kaur et al. (2012). Purified
ubiquinones were dissolved in 2-propanol and analysed
phospholipids and glycolipids (Fig. S2). Polyamines were
extracted as described by Madhubala (1997) and analysed
Polar lipid analysis of strain LL03T exhibited the presence
of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylgly-
cerol, phosphatidylcholine, sphingoglycolipid, unknown
phospholipids and glycolipids (Fig. S2). Polyamines were
extracted as described by Madhubala (1997) and analysed
by reverse-phase TLC according to Collins & Jones (1980).
Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequence data showing the evolutionary relationship
of strain LL03T and members of genus Sphingobium. The tree was constructed by using neighbour-joining method of TREECON W
software and the rooting was done using Z. mobilis ATCC 10988T as an outgroup. Bar, 0.02 nt substitutions per nt position.
The GenBank accession number for the 16S rRNA gene sequence of each strain is shown in parentheses. Asterisks indicate
the branches that were also found using the maximum-parsimony and neighbour-joining methods of PHYLP 3.69. Numbers at
nodes represent the bootstrap values based on 1000 resamplings.
next step, quinones were extracted according to the
protocol described by Kaur et al. (2012). Purified
ubiquinones were dissolved in 2-propanol and analysed
by reverse-phase TLC according to Collins & Jones (1980).
Polar lipid analysis of strain LL03T was carried out by two-
dimensional TLC as described by Bligh & Dyer (1959).
Polar lipid analysis of strain LL03T exhibited the presence
of phosphatidylethanolamine, phosphatidylmonomethyl-
ethanolamine, phosphatidylglycerol, diphosphatidylgly-
cerol, phosphatidylcholine, sphingoglycolipid, unknown
phospholipids and glycolipids (Fig. S2). Polyamines were
extracted as described by Madhubala (1997) and analysed
by one-dimensional TLC. Ten microlitres of the extracted
sample was applied on a TLC plate (silica gel 60 F254,
20 cm; Merck, 1.05554.0007) and running solvent
was cyclohexane/ethylacetate (3:2). Spermidine was
identified as the major polyamine in strain LL03T, which is
characteristic of the genus Sphingobium (Busse et al.,
1999; Takeuchi et al., 2001).

The DNA G+C content of strain LL03T calculated by the
method described by Gonzalez & Saiz-Jimenez (2002)
using Applied Biosystems 7500 Real-Time PCR system, was
found to be 67.0 mol%. Motility of the organism was also
checked on motility agar, the strain was found to be non-
motile. The colony morphology of strain LL03T was
studied on Luria–Bertani agar (LB), nutrient agar (NA)
Table 1. Cellular fatty acids of strain LL03T and closely related members of the genus Sphingobium

Data were obtained in this study (under similar conditions) unless indicated. Strains: 1, strain LL03T; 2, Sphingobium wenxiniae JZ-1T; 3, Sphingobium faniae JZ-2T; 4, Sphingobium cloacae S-3T; 5, Sphingobium amiense YT; 6, Sphingobium vernicposito VC-230T; 7, Sphingobium quisquiliarum P25T; and 8, Sphingobium chlorophenolicum ATCC 33790T. Values < 1% have been designated TR (trace).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.0</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>16:0</td>
<td>14.3</td>
<td>24.3</td>
<td>3.5</td>
<td>4.6</td>
<td>4.6</td>
<td>6.5</td>
<td>7.8</td>
<td>8.4</td>
</tr>
<tr>
<td>18:0</td>
<td>3.3</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>16:1o5c</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.0</td>
<td>1.7</td>
<td>3.8</td>
<td>3.8</td>
<td>2.9</td>
</tr>
<tr>
<td>17:1o6c</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.8</td>
<td>14.6</td>
<td>6.0</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>17:1o8c</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.7</td>
<td>1.0</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>18:1o5c</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>6.3</td>
<td>3.7</td>
<td>5.0</td>
<td>11.0</td>
<td>5.8</td>
</tr>
<tr>
<td>18:1o7c</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.5</td>
<td>2.6</td>
<td>2.1</td>
<td>2.6</td>
<td>4.1</td>
</tr>
<tr>
<td>14:0 2-OH</td>
<td>8.4</td>
<td>7.1</td>
<td>11.0</td>
<td>31.6</td>
<td>7.2</td>
<td>9.9</td>
<td>2.9</td>
<td>14.6</td>
</tr>
<tr>
<td>12:0 2-OH</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>5.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>TR</td>
</tr>
<tr>
<td>Summed feature:*</td>
<td>19.0</td>
<td>34.4</td>
<td>12.6</td>
<td>2.7</td>
<td>8.0</td>
<td>13.8</td>
<td>5.3</td>
<td>8.5</td>
</tr>
</tbody>
</table>

*Summed feature 3 consists of 16:1o7c and/or 16:1o6c and summed feature 8 consists of 18:1o7c and/or 18:1o6c.

Table 2. Differential physiological characteristics of strain LL03T and closely related members of the genus Sphingobium

Data were obtained in this study (under similar conditions) unless indicated. Strains: 1, LL03T; 2, S. wenxiniae JZ-1T; 3, S. faniae JZ-2T; 4, S. cloacae S-3T; 5, S. amiense YT; 6, S. vernicposito VC-230T; 7, S. quisquiliarum P25T; and 8, S. chlorophenolicum ATCC 33790T.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>White</td>
<td>Yellow</td>
<td>Pale yellow</td>
<td>Cream</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>HCH degradation</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>--</td>
</tr>
<tr>
<td>Urease</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Tween 80</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Tween 20</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Cascin*</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Indole</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Maltose</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*Sodium salt.
was tested using a HiMedia DNase agar plate. Several morphological, chemotaxonomic and biochemical differences were found between strain LL03\(^T\) and its nearest neighbour \(S.\) wenxiiae \(YZ-1\) (Table 2). Some of these include: the strain is oxidase and catalase-positive and can reduce nitrate to nitrite; hydrolyzes aesculin and utilizes D-mannose and maltose as carbon source, whereas the results with \(YZ-1\) are negative for all these tests. Further, the strain cannot utilize citrate; hydrolyse Tween 20, Tween 80 or casein (sodium salt), whereas the results with \(YZ-1\) were positive for all these tests. Based on the results obtained from morphological, physiological and biochemical analysis, it is proposed that strain LL03\(^T\) represents a novel species of the genus \(Sphingobium\), for which the name \(Sphingobium\) baderi sp. nov. is proposed.

### Description of \(Sphingobium baderi\) sp. nov.

\(Sphingobium baderi\) (ba’de.ri N.L. gen. masc. n. baderi of Bader, named for Alfred Bader, a Czech-born chemist and a co-founder of Aldrich Chemical Company).

Cells are Gram-stain-negative and rod-shaped, and produce white-coloured colonies. Growth occurs on LB, NA, R2A and TSA. The optimum temperature, pH and concentration of NaCl for growth are 28°C, 6–8 and 2–3%, respectively. Catalase and oxidase tests are positive. Nitrate is reduced to nitrite. Cannot hydrolyse Tween 20, starch or xanthine. Indole production is negative. Resistant to penicillin, streptomycin, nalidixic acid and ampicillin, but is sensitive to tetracycline, gentamicin, rifampicin, ciprofloxacin, vancomycin, chloramphenicol, kanamycin, polymyxin B and oxytetracycline. Decreases \(\alpha\), \(\gamma\) and \(\delta\)-HCH isomers in liquid culture. Predominant respiratory quinone is ubiquinone Q-10. Major polar lipids are sphingoglycolipid, phosphatidylethanolamine, phosphatidimonomethylethanolamine, phosphatidylcholine, diphosphatidylglycerol and phosphatidylethanolamine. Major polyamine is spermidine. The predominant fatty acids are 16:0, summed feature 3 (16:1 \(\omega\)7c and/or 16:1 \(\omega\)6c), summed feature 8 (18:1 \(\omega\)7c and/or 18:1 \(\omega\)6c) and 14:0 2-OH.

The type strain is LL03\(^T\) (=CCM 7981\(^T\)=DSM 25433\(^T\)), isolated from HCH-contaminated soil at Spolana Neratovice, Czech Republic. The DNA G+C content of the type strain is 67.0 mol%.

### Acknowledgements

This research was supported by funds from Department of Biotechnology (DBT), National Bureau of Agriculturally Important Microorganisms (NBAIM, ICAR), DU/DST-PURSE Grant, Government of India, the Grant Agency of the Czech Republic (203/08/0114 and P202/10/1435), the Grant Agency of the Czech Academy of Sciences (IAA401630901) and the European Regional Development Fund (CZ.1.05/2.1.00/01.0001 and CZ.1.05/1.1.00/02.0123). J.K. and N.N. gratefully acknowledge Council for Scientific and Industrial Research (CSIR) for providing fellowships. We would also like to thank J.P. Euzéby (Ecole Nationale Veterinaire, Toulouse, France) for etymological advice and Dr Jian He (College of life science of Nanjing Agricultural University, China) for providing \(Sphingobium\) wenxiiae \(YZ-1\) and \(Sphingobium\) faniae \(YZ-2\).

### References


Christensen, W. B. (1946). Urea decomposition as means of differentiating \(Proteus\) and \(Paracolon\) cultures from each other and from \(Salmonella\) and \(Shigella\). J Bacteriol 52, 461–466.


