**Rhizobium rosettiformans** sp. nov., isolated from a hexachlorocyclohexane dump site, and reclassification of *Blastobacter aggregatus*

Hirsch and Müller 1986 as *Rhizobium aggregatum* comb. nov.

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A Gram-negative, rod-shaped, motile, aerobic bacterial strain, W3\(^{T}\), was isolated from hexachlorocyclohexane (HCH)-contaminated groundwater from Lucknow, India, and its taxonomic position was determined using a polyphasic approach. Strain W3\(^{T}\) shared highest 16S rRNA gene sequence similarity of 97.8% with *Rhizobium selenitireducens* B1\(^{T}\), followed by *Rhizobium daejeonense* L61\(^{T}\) (97.7%), *Rhizobium radiobacter* ATCC 19358\(^{T}\) (97.5%) and *Blastobacter aggregatus* IFAM 1003\(^{T}\) (97.2%). Strain W3\(^{T}\) formed a monophyletic clade with *Blastobacter aggregatus* IFAM 1003\(^{T}\) (=DSM 1111\(^{T}\)) in the cluster of species of the genus *Rhizobium*. Phylogenetic analyses of strain W3\(^{T}\) using *atpD* and *recA* gene sequences confirmed the phylogenetic arrangements obtained by using 16S rRNA gene sequences. Hence, the taxonomic characterization of *B. aggregatus* DSM 1111\(^{T}\) was also undertaken. Strains W3\(^{T}\) and *B. aggregatus* DSM 1111\(^{T}\) contained summed feature 8 (18:1\(\omega_7\)c and/or 18:1\(\omega_6\)c; 65.4 and 70.8%, respectively) as the major fatty acid, characteristic of the genus *Rhizobium*. DNA–DNA relatedness of strain W3\(^{T}\) with *Rhizobium selenitireducens* LMG 24075\(^{T}\), *Rhizobium daejeonense* DSM 17795\(^{T}\), *Rhizobium radiobacter* DSM 30147\(^{T}\) and *B. aggregatus* DSM 1111\(^{T}\) was 42, 34, 30 and 34%, respectively. The DNA G+C contents of strain W3\(^{T}\) and *B. aggregatus* DSM 1111\(^{T}\) were 62.3 and 62.7 mol%, respectively. A *nifH* gene encoding dinitrogenase reductase was detected in strain W3\(^{T}\) but not in *B. aggregatus* DSM 1111\(^{T}\). Based on the results obtained by phylogenetic and chemotaxonomic analyses, phenotypic characterization and DNA–DNA hybridization, it is concluded that strain W3\(^{T}\) represents a novel species of the genus *Rhizobium*, for which the name *Rhizobium rosettiformans* sp. nov. is proposed (type strain W3\(^{T}\) =CCM 7583\(^{T}\) =MTCC 9454\(^{T}\)). It is also proposed that *Blastobacter aggregatus* Hirsch and Müller 1986 be transferred to the genus *Rhizobium* as *Rhizobium aggregatum* comb. nov. (type strain IFAM 1003\(^{T}\) =DSM 1111\(^{T}\) =ATCC 43293\(^{T}\)).

**Abbreviation:** HCH, hexachlorocyclohexane.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *nifH*, *atpD* and *recA* gene sequences of strain W3\(^{T}\) are EU781656, GU241353, GU559878 and GU562963, respectively. Those for the *atpD* gene sequences of *Blastobacter aggregatus* DSM 1111\(^{T}\), *Rhizobium daejeonense* DSM 17795\(^{T}\) and *Rhizobium selenitireducens* LMG 24075\(^{T}\) are GU559879–GU559882, respectively, and those for the *recA* gene sequences of *B. aggregatus* DSM 1111\(^{T}\), *R. daejeonense* DSM 17795\(^{T}\), *R. radiobacter* DSM 30147\(^{T}\) and *R. selenitireducens* LMG 24075\(^{T}\) are GU559874–GU559877, respectively.

A supplementary figure and a supplementary table are available with the online version of this paper.

In the present study, the objective was to classify a bacterial strain, W3\(^{T}\), which was isolated from a water sample drawn from a hand pump situated in a hexachlorocyclohexane (HCH) dumpsite. The water sample was plated on Luria–Bertani (LB) agar. A cream-coloured colony that appeared within 48 h of incubation at 28 °C was picked and purified by repeated streaking on LB agar. Phylogenetic trees reconstructed using 16S rRNA, *atpD* and *recA* gene sequences showed a close relationship of strain W3\(^{T}\) with *Blastobacter aggregatus* IFAM 1003\(^{T}\) in the *Rhizobium* cluster. Thus, the taxonomic position of *B. aggregatus* was also reassessed.

Species of the genus *Blastobacter* Zavarzin 1961, belonging to the order *Rhizobiales* and family *Bradyrhizobiaceae*, are rod-shaped, commonly aquatic (especially freshwater), budding, rosette-forming and do not fix nitrogen (Rothe *et al.*, 1987; Sly & Hugenholtz, 2005; Balachandar *et al.*, 2011).
2007). The genus is in fact polyphyletic, with a high degree of heterogeneity with respect to its phenotypic (Trotsenko et al., 1989; Sittig & Hirsch, 1992), chemotaxonomic and molecular characteristics (Rothe et al., 1987; Willems & Collins, 1992; Hugenholtz et al., 1994). Two of the five species of the genus *Rhizobium* with validly published names have been transferred subsequently to other genera: *Blastobacter natatorius* to *Blastomonas* (Sly & Cahill, 1997) and *Blastobacter denitrificans* to *Bradyrhizobium* (van Berkum et al., 2006), although the new combination ‘*Bradyrhizobium denitrificans*’ has not been validly published. *B. aggregatus* IFAM 1003\(^T\) showed a close relationship with *Rhizobium* species on the basis of 16S rRNA gene cataloguing (Rothe et al., 1987), and the need to revise the taxonomy of genus *Blastobacter* has also been suggested (Sly & Cahill, 1997; Doronina & Trotsenko, 2003). On the other hand, species of the genus *Rhizobium* Frank 1889, belonging to the class *Alphaproteobacteria*, order *Rhizobiales*, family *Rhizobiaceae*, are rod-shaped and include free-living species as well as those that form nitrogen-fixing symbioses with roots of leguminous plants. These symbioses are of considerable importance in agriculture and reduce the need for chemical fertilizers. Rhizobia are commonly found in soil, but have also been isolated from water (Zurdo-Piñeiro et al., 2004), non-legume plants (Peng et al., 2008) and bioreactors (Hunter et al., 2007; Quan et al., 2005). Fatty acids common to all species of the genus *Rhizobium* include 16:0, 18:0, summed feature 2 (one or more of 12:0 aldehyde, iso-16:1 I and 14:0 3-OH), summed feature 3 (16:1ω7c and/or 16:1ω6c) and summed feature 8 (18:1ω7c and/or 18:1ω6c) (Tighe et al., 2000).

The reference strains used in this study were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) (*Blastobacter aggregatus* DSM 1111\(^T\), *Rhizobium daejeonense* DSM 17795\(^T\) and *Rhizobium radiobacter* DSM 30147\(^T\)) and the Laboratorium voor Microbiologie, Universiteit Gent (*Rhizobium selenitireducens* LMG 24075\(^T\)).

Gram staining was performed using a Gram stain kit (HiMedia). Colony morphology was examined on LB agar plates under a light microscope (Mycopt 200) after incubation at 28 °C for 48 h. Cells in the early exponential growth phase were used for detecting flagella using transmission electron microscopy as described by Kumar et al. (2008).

Genomic DNA was isolated as described by Sambrook et al. (1989). A nearly complete (1406 bp) 16S rRNA gene sequence of strain W3\(^T\) was obtained according to the method described by Kumar et al. (2008). For phylogenetic analyses, 16S rRNA gene sequences of strains of species with validly published names that are closely related to strain W3\(^T\) and *B. aggregatus* IFAM 1003\(^T\) (Fig. 1) were retrieved from the GenBank database at NCBI (http://blast.ncbi.nlm.nih.gov/blast.cgi; Altschul et al., 1997). The selected sequences were aligned using the CLUSTAL_X software, version 1.81b (Thompson et al., 1997), and terminal nucleotides not common to all sequences were trimmed. The evolutionary distance matrix was calculated using the distance model of Jukes & Cantor (1969) within the TREECONW software package, version 1.3b (Van de Peer & De Wachter, 1994). A phylogenetic tree (Fig. 1) was reconstructed using the neighbour-joining method of Saitou & Nei (1987) and the resultant tree topology was evaluated using bootstrap analysis of 1000 replications. Similar tree topologies were obtained using Kimura’s two-parameter model in TREECONW and a DNA parsimony method using PHYLIP, version 3.5 (Felsenstein, 1993) (not shown). The results indicated that strain W3\(^T\) and *B. aggregatus* IFAM 1003\(^T\) grouped with representatives of the genus *Rhizobium*, with clear separation of *B. aggregatus* IFAM 1003\(^T\) from other species of the genus *Blastobacter* with validly published names. Strain W3\(^T\) shared 97.8 % 16S rRNA gene sequence similarity with *R. selenitireducens* B1\(^T\), followed by *R. daejeonense* L61\(^T\) (97.7 %), *R. radiobacter* ATCC 19358\(^T\) (97.5 %) and *B. aggregatus* IFAM 1003\(^T\) (97.2 %). Likewise, *B. aggregatus* IFAM 1003\(^T\) shared 95.6 % 16S rRNA gene sequence similarity with *R. daejeonense* L61\(^T\) and *Blastobacter capsulatus* IFAM 1005\(^T\), followed by *R. selenitireducens* B1\(^T\) (95.3 %) and *R. radiobacter* ATCC 19358\(^T\) (95.0 %).

To clarify further the taxonomic status of strain W3\(^T\) and *B. aggregatus* DSM 1111\(^T\), two housekeeping genes, *atpD* and *recA*, were amplified and sequenced as described by Gaunt et al. (2001). Partial *atpD* and *recA* gene sequences of strain W3\(^T\), *B. aggregatus* DSM 1111\(^T\), *R. selenitireducens* LMG 24075\(^T\), *R. daejeonense* DSM 17795\(^T\) and *R. radiobacter* DSM 30147\(^T\) were obtained. Phylogenetic trees based on *atpD* (Fig. 2) and *recA* (Fig. 3) gene sequences were reconstructed by using the neighbour-joining method of Saitou & Nei (1987). Strain W3\(^T\) and *B. aggregatus* DSM 1111\(^T\) exhibited 88.6–94.5 % *atpD* gene sequence similarity and 84.5–89.2 % *recA* gene sequence similarity to the reference *Rhizobium* strains used in this study.

To sum up, phylogenetic analyses based on ATP synthase β (*atpD*) and DNA recombinase A (*recA*) gene sequences corroborated the 16S rRNA gene sequence-based phylogeny, thereby providing strong evidence that strain W3\(^T\) and *B. aggregatus* DSM 1111\(^T\) belong to the genus *Rhizobium*.

Symbiotic properties are important characteristics of rhizobia; hence the presence of symbiotic genes in strain W3\(^T\) and *B. aggregatus* DSM 1111\(^T\) was examined by amplifying *nifH* (Poly et al., 2001), *nodA* (Haukka et al., 1998), *nodC* (Lagueur et al., 2001) and *nodD* (Rivas et al., 2002). A partial *nifH* gene sequence (344 bp) from strain W3\(^T\) showed highest sequence similarity with that of *Azohydromonas lata* IAM 12599\(^T\) (93.8 %) followed by *Sinorhizobium* sp. TJ170 (91.8 %) and *Azohydromonas australica* IAM 12664\(^T\) (91.0 %). However, no *nifH* gene could be detected in *B. aggregatus* DSM 1111\(^T\). None of the...
three nod genes could be detected in strain W3\textsuperscript{T} or \textit{B. aggregatus} DSM 1111\textsuperscript{T}. Fatty acid methyl ester analysis of strain W3\textsuperscript{T}, \textit{B. aggregatus} DSM 1111\textsuperscript{T}, \textit{R. selenitireducens} LMG 24075\textsuperscript{T}, \textit{R. daejeonense} DSM 30147\textsuperscript{T} and \textit{R. radiobacter} DSM 30147\textsuperscript{T} was performed at Disha Institute of Biotechnology Pvt. Ltd (Ahmedabad, India) using the method described by Miller (1982) and Kuykendall et al. (1988). The major fatty acid in strain W3\textsuperscript{T} and \textit{B. aggregatus} DSM 1111\textsuperscript{T} was summed feature 8 (18:1\textit{v}\textsubscript{7}c and/or 18:1\textit{v}\textsubscript{6}c). Overall, the fatty acid profiles of strain W3\textsuperscript{T} and \textit{B. aggregatus} DSM 1111\textsuperscript{T} (Supplementary Table S1, available in IJSEM Online) were in accordance with those of members of the genus \textit{Rhizobium} (Hunter et al., 2007; Tighe et al., 2000), further supporting the placement of strain W3\textsuperscript{T} and \textit{B. aggregatus} DSM 1111\textsuperscript{T} in the genus \textit{Rhizobium}.

DNA–DNA hybridization is considered to be a standard method to affirm species delineation (Wayne et al., 1987). Hence, DNA–DNA hybridization studies were conducted between strain W3\textsuperscript{T}, \textit{B. aggregatus} DSM 1111\textsuperscript{T}, \textit{R. selenitireducens} LMG 24075\textsuperscript{T}, \textit{R. daejeonense} DSM 17795\textsuperscript{T} and \textit{R. radiobacter} DSM 30147\textsuperscript{T} as described by Tourova & Antonov (1987) and Bala et al. (2004). DNA (10 \mu g) from 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 and the DNA probe for each strain was labelled with \[^{32}\text{P}]	ext{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 \(b\)-scintillation counter (Perkin Elmer) and the hybridization values obtained were expressed as percentages of the amount of probe bound relative to the homologous reaction. Since low 16S rRNA gene sequence similarities (<97%) were found between \textit{B. aggregatus} IFAM 1003\textsuperscript{T} and type strains of other species of the genera \textit{Rhizobium} and \textit{Blastobacter} with validly published names, DNA–DNA hybridization studies were not performed between \textit{B. aggregatus} DSM 1111\textsuperscript{T} and closely related type strains. The DNA–DNA relatedness of strain W3\textsuperscript{T} with \textit{R. selenitireducens} LMG 24075\textsuperscript{T}, \textit{R. daejeonense} DSM 17795\textsuperscript{T} and \textit{R. radiobacter} DSM 30147\textsuperscript{T} as described by Tourova & Antonov (1987) and Bala et al. (2004).
17795\textsuperscript{T}, R. radiobacter DSM 30147\textsuperscript{T} and B. aggregatus DSM 1111\textsuperscript{T} was 42, 34, 30 and 34 %, respectively. These values are clearly below the recommended threshold value for delineation of genospecies (Stackebrandt & Goebel, 1994), indicating that strain W3\textsuperscript{T} represents a novel species of the genus Rhizobium.

The DNA G + C contents of strain W3\textsuperscript{T}, R. selenitireducens LMG 24075\textsuperscript{T}, R. daejeonense DSM 17795\textsuperscript{T}, R. radiobacter DSM 30147\textsuperscript{T} and B. aggregatus DSM 1111\textsuperscript{T} were estimated using the procedure of Gonzalez & Saiz-Jimenez (2002) (Table 1). The DNA G + C contents of strain W3\textsuperscript{T} and B. aggregatus DSM 1111\textsuperscript{T} were found to be 62.3 and...
Table 1. Differential characteristics of strain W3\textsuperscript{T}, *B. aggregatus* DSM 1111\textsuperscript{T} and type strains of related *Rhizobium* species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>4</th>
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<th>6</th>
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<tr>
<td>Cell morphology</td>
<td>Rod</td>
<td>Rod*</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rods, often bent</td>
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<tr>
<td>Flagellation/motility</td>
<td>+</td>
<td>+*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Colony colour</td>
<td>Cream</td>
<td>Cream</td>
<td>Opaque white</td>
<td>Cream</td>
<td>Light beige</td>
<td>White\textsuperscript{\dagger}</td>
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<td>Growth in:</td>
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<tr>
<td>2% NaCl</td>
<td>+</td>
<td>+*</td>
<td>−</td>
<td>+</td>
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<tr>
<td>3% NaCl</td>
<td>+</td>
<td>+*</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Citrate utilization</td>
<td>+</td>
<td>−*</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Nitrate reduction</td>
<td>+</td>
<td>−*</td>
<td>+</td>
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<tr>
<td>Urease</td>
<td>+</td>
<td>+*</td>
<td>−</td>
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<td>β-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Utilization as carbon source of:</td>
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<tr>
<td>Lactose</td>
<td>−</td>
<td>+*</td>
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<td>D-Fructose</td>
<td>−</td>
<td>−*</td>
<td>−$</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Raffinose</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>ND</td>
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<td>D-Mannitol</td>
<td>+\textsuperscript{e}</td>
<td>+*</td>
<td>−</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Adonitol</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>ND</td>
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<tr>
<td>D-Galactose</td>
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<td>−*</td>
<td>−$</td>
<td>+</td>
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<td>+</td>
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<td>Utilization as nitrogen source of:</td>
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<tr>
<td>L-Ornithine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>L-Proline</td>
<td>+</td>
<td>−*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Serine</td>
<td>+</td>
<td>−*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Reduction of methylene blue</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Presence of mfbH</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>62.3,62.7 (60.4±0.4)\textsuperscript{d}</td>
<td>62.9 (60.8)\textsuperscript{e}</td>
<td>63.6 (60.1–60.9)\textsuperscript{f}</td>
<td>64.3 (57–63)\textsuperscript{f}</td>
<td>58.9</td>
<td></td>
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</table>

\*Data in agreement with results reported by Hirsch & Müller (1985).
\textsuperscript{†}Data taken from: a, Sly (1985); b, Sly & Hugenholtz (2005); c, Quan et al. (2005); d, Hirsch & Müller (1985); e, Hunter et al. (2007); f, Sawada et al. (1993).
§Reported as negative by Hunter et al. (2007).
\textsuperscript{\dagger}Reported as positive by Hunter et al. (2007).

62.7 mol\%, respectively, well within the range reported for other *Rhizobium* species (Jordan, 1984).

All phenotypic tests for strain W3\textsuperscript{T} and *B. aggregatus* DSM 1111\textsuperscript{T} were performed at 28 °C unless stated otherwise. The ability of the strains to grow at 4–45 °C, pH 4–12 and 0–5% (w/v) NaCl was determined as described by Arden Jones et al. (1979). Strain W3\textsuperscript{T} showed rich growth on LB agar and tryptic soy agar (TSA) but slow growth was seen on nutrient agar (NA), whereas *B. aggregatus* DSM 1111\textsuperscript{T} showed good growth on LB agar but slow growth on TSA and NA. Motility of the two cultures was checked on motility agar (Farmer, 1999). Like *B. aggregatus* DSM 1111\textsuperscript{T}, strain W3\textsuperscript{T} was found to form rosettes (Supplementary Fig. S1). The two strains were tested for oxidase activity using Oxidase discs (HiMedia). Catalase activity was determined by bubble production in a 3% (v/v) hydrogen peroxide solution (Merck) as described by McCarthy & Cross (1984). The ability of the strains to produce melanin was checked as described by Cubo et al. (1988). Urease activity was tested using the method of Christensen (1946). Utilization of different carbohydrates was detected by using the HiCarbohydrate kit (HiMedia). Tests for indole production, nitrate reduction and the Voges–Proskauer reaction were performed as described by Smibert & Krieg (1994) and Prakash et al. (2007). Hydrolysis of gelatin, starch and aesculin was assessed as described by Cowan & Steel (1965). Citrate utilization was tested on Simmons’ citrate agar (Simmons, 1926). Cellulase activity was checked on CEA medium (0.7%...
CM-cellulose, 0.3 % yeast extract, 2.5 % agar) after 5 days of incubation at 28 °C (Garcia-Fraile et al., 2007). The ability of the strains to reduce methylene blue was tested as described by Gao et al. (1994). The ability of the strains to produce H2S was tested using TSI medium (1 %: 3 g beef extract, 3 g yeast extract, 20 g meat peptic digest, 1 g glucose, 10 g lactose, 10 g sucrose, 0.3 g ferric citrate, 5 g NaCl, 0.3 g sodium sulfate, 12 g agar and 0.025 g phenol red; pH 7.4). Differential results of biochemical tests with the closest relatives of strain W3T and B. aggregatum DSM 1111T are given in Table 1.

While strain W3T was found to be positive for utilization of citrate, reduction of nitrate and methylene blue, B. aggregatum DSM 1111T was negative for these tests. Both strains were found to be positive for hydrolysis of aesculin and activities of catalase, oxidase and urease. Based on the results obtained, it is proposed that strain W3T represents a novel species of the genus Rhizobium, for which the name Rhizobium rosettiformans sp. nov. is proposed. It is also proposed that Blastobacter aggregatus be transferred to the genus Rhizobium as Rhizobium aggregatum comb. nov.

**Description of Rhizobium rosettiformans sp. nov.**


Gram-negative, aerobic, motile, rod-shaped (0.6–0.7 × 1.3–1.5 μm) and rosette-forming. Colonies are cream-coloured, circular with entire margins and 0.5–1.2 mm in diameter when grown on LB agar at 28 °C for 48 h. The presence of a single flagellum is revealed by transmission electron microscopy. Rich growth on TSA but slow growth on NA. Grows at 25–40 °C, pH 6–10 and 0–3 % (w/v) NaCl. Optimum conditions are 28 °C, pH 7.0 and 1.0 % NaCl. Catalase- and oxidase-positive. Positive for hydrolysis of urease, β-galactosidase activity and utilization of D-glucose, maltose, D-mannitol, L-ornithine, L-proline and L-serine. Utilizes citrate and nitrate is reduced. Can grow in medium supplemented with 0.1 % methylene blue. Does not hydrolyse starch or gelatin and nitrate is not reduced. Negative for cellulase activity, indole and melanin production and utilization of D-fructose, raffinose, D-galactose, L-proline and L-serine. Negative for utilization of citrate. Cannot grow in medium supplemented with 0.1 % methylene blue. Voges–Proskauer reaction is negative. H2S is not produced. Sensitive to chloramphenicol (30 μg), rifampicin (5 μg) and tetracycline (30 μg). Less sensitive to gentamicin (10 μg) and vancomycin (30 μg) and resistant to kanamycin (30 μg). The major cellular fatty acid is summed feature 8 (18:1o7c and/or 18:1o6c), followed by summed feature 2 (one or more of 12:0 aldehyde, iso-16:1 I and 14:0 3-OH), 18:0 3-OH, 18:0, 16:0, 11-methyl 18:1o7c, summed feature 3 (16:1o7c and/or 16:1o6c), iso-18:0 and 17:0. Traces (<1 %) of the following fatty acids are also present: 12:0, 14:0, 17:1o6c, 17:1o8c, 18:0o5c, 20:1o7c, iso-14:0 3-OH, 15:0 3-OH, 16:0 3-OH, 17:0 3-OH and summed feature 7 (one or more of 19:1o6c, 19:1o7c and 19:0 cyclo). A *nifH* gene encoding a component of the nitrogenase complex is detected. The DNA G+C content of the type strain is 62.3 mol%.

The type strain is W3T (=CCM 7583T =MTCC 9454T), isolated from an HCH dump site in India.

**Description of Rhizobium aggregatum comb. nov.**


Basonym: Blastobacter aggregatus Hirsch and Müller 1986. Cells are Gram-negative, aerobic, motile, rod-shaped (0.5–0.6 × 1.8–2.1 μm) and rosette-forming. Colonies are cream-coloured, circular with entire margins and 0.5–1.0 mm in diameter when grown on LB agar at 28 °C for 48 h. The presence of a single flagellum is revealed by transmission electron microscopy. Good growth occurs on LB agar but growth is slow on TSA and NA. Grows at 25–40 °C, pH 6–10 and 0–3 % (w/v) NaCl. Optimum conditions are 28 °C, pH 7.0 and 1.0 % NaCl. Positive for β-galactosidase, urease, catalase and oxidase activities. Positive for utilization of D-glucose, maltose, lactose, D-mannitol, adonitol and L-ornithine. Does not hydrolyse starch or gelatin and nitrate is not reduced. Negative for cellulase activity, indole and melanin production and utilization of D-fructose, raffinose, D-galactose, L-proline and L-serine. Negative for utilization of citrate. Cannot grow in medium supplemented with 0.1 % methylene blue. Voges–Proskauer reaction is negative. H2S is not produced. Sensitive to chloramphenicol (30 μg), rifampicin (5 μg) and tetracycline (30 μg). Less sensitive to gentamicin (10 μg) and vancomycin (30 μg) and resistant to kanamycin (30 μg). The major cellular fatty acid is summed feature 8 (18:1o7c and/or 18:1o6c), followed by summed feature 2 (one or more of 12:0 aldehyde, iso-16:1 I and 14:0 3-OH), 11-methyl 18:1o7c, 16:0, 18:0, 18:0 3-OH, summed feature 3 (16:1o7c and/or 16:1o6c) and iso-18:0. Traces (<1 %) of the following fatty acids are also present: 12:0, 14:0, 17:0, 17:1o6c, 17:1o8c, 18:0o5c, 20:1o7c, iso-14:0 3-OH, 15:0 3-OH, 16:0 3-OH, 17:0 3-OH and summed feature 7 (one or more of 19:1o6c, 19:1o7c and 19:0 cyclo). The *nifH* gene cannot be detected in the type strain. The DNA G+C content of the type strain is 62.7 mol%.

The type strain is IFAM 1003T (=DSM 1111T =ATCC 43293T), isolated from Lake Höftsee (Holstein, Germany).

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