

## *Schlegelella*

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**2. KEYWORDS:** *Schlegelella*; chemo-organotroph; polyhydroxyalkanoates (PHA) accumulation; PHA biodegradation; abietane resin acids degradation; antitumour glidobactins production.

23 **3. ABSTRACT:**

24 **Short rods** 0.8-2.8  $\mu\text{m}$  long and 0.4-0.6  $\mu\text{m}$  wide. **Motile. Gram-negative.** Nonsporulating.  
25 **Aerobe.** Moderate thermophilic or mesophilic, with optimal growth occurring at 45-50 °C or  
26 30-37°C, respectively. **Chemo-organotroph.** Accumulates polyhydroxyalkanoates (PHA).  
27 Catalase and cytochrome *c* oxidase variable. Major fatty acids are C<sub>16:0</sub> and cyclo-C<sub>17:0</sub> or  
28 C<sub>16:1</sub>  $\omega$ 7*c* and/or C<sub>16:1</sub>  $\omega$ 6*c*, depending on the species. The type species is *Schlegelella*  
29 *thermodepolymerans*. *Schlegelella aquatica* and *Schlegelella brevitalia* are two other validly  
30 named species of this genus.

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32 **4. DEFINING PUBLICATION:**

33 *Schlegelella*, Elbanna, Lütke-Eversloh, Van Trappen, Mergaert, Swings and Steinbüchel 2003,  
34 1167<sup>VP</sup> *emend.* Lütke-Eversloh, Elbanna, Cnockaert, Mergaert, Swings, Manaia and  
35 Steinbüchel 2004, 1934.

36

37 **5. ETYMOLOGY:**

38 *Schlegelella* [Schle.gel.el'la. L. fem. dim. ending *-ella*; N.L. fem. n. *Schlegelella* named in  
39 honour of H. G. Schlegel, a pioneer in PHA research].

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41 **6. GENERIC DEFINITION:**

42 **Short rods** 0.8-2.8  $\mu\text{m}$  long and 0.4-0.6  $\mu\text{m}$  wide. **Motile. Gram-negative.** Nonsporulating.  
43 **Aerobe.** Moderate thermophilic or mesophilic, with optimal growth occurring at 45-50 °C or  
44 30-37°C, respectively. **Chemo-organotroph.** Accumulates polyhydroxyalkanoates (PHA).  
45 Catalase and cytochrome *c* oxidase variable. Major fatty acids are C<sub>16:0</sub> and cyclo-C<sub>17:0</sub> or

46 C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c, depending on the species. The type species is *Schlegelella*  
47 *thermodepolymerans*. *Schlegelella aquatica* and *Schlegelella brevitalea* are two other validly  
48 named species of this genus.

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50 The DNA G+C content (mol %) is 69.2-70.0 (HPLC) or 67.5-70.3 (genome analysis,  
51 GenBank).

52

53 Type species: *Schlegelella thermodepolymerans*, Elbanna, Lütke-Eversloh, Van Trappen,  
54 Mergaert, Swings and Steinbüchel 2003, 1167<sup>VP</sup>

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56 Number of species with validated names: 3.

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## 58 **7. FAMILY CLASSIFICATION:**

59 *Comamonadaceae* (fbm00182)

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## 61 **8. FURTHER DESCRIPTIVE INFORMATION:**

### 62 **8.1. *Schlegelella* strains**

63 Three species are validly named within the genus *Schlegelella*: *Schlegelella*  
64 *thermodepolymerans* (Elbanna et al., 2003), *Schlegelella aquatica* (Chou et al., 2006) and  
65 *Schlegelella brevitalea* (Tang et al., 2019). *Schlegelella thermodepolymerans*, the type species  
66 of this genus, was described based on the characterization of strain K14<sup>T</sup>, isolated from  
67 activated sludge (Elbanna et al., 2003) and strain DhA-71, isolated from municipal compost

68 (Yu and Mohn, 1999), which showed 94% DNA–DNA hybridization reassociation levels  
69 (Elbanna et al., 2003). A later synonym, *Caenibacterium thermophilum* represented by strain  
70 N2-680, isolated from a thermophilic aerobic digester of a domestic wastewater-treatment  
71 plant, was proposed soon after the description of *Schlegelella thermodepolymerans* (Manaia et  
72 al., 2003). Further studies revealed that strain N2-680 shares 99.8-99.6% 16S rRNA gene  
73 sequence similarity and 97–98% DNA–DNA hybridization reassociation levels with strains  
74 K14<sup>T</sup> and DhA-71 (Lütke-Eversloh et al., 2004). These results demonstrated that  
75 *Caenibacterium thermophilum* was a later synonym of *Schlegelella thermodepolymerans*  
76 (Lütke-Eversloh et al., 2004).

77 Both *Schlegelella aquatica* and *Schlegelella brevitalea* were described based on the  
78 characterization of a single strain. Strain wcf1<sup>T</sup>, isolated from a hot spring, represents  
79 *Schlegelella aquatica* and strain K481-B101<sup>T</sup>=DSM 7029<sup>T</sup>=ATCC 53080<sup>T</sup>, isolated from soil,  
80 was named as *Schlegelella brevitalea* (Chou et al., 2006, Tang et al., 2019). Originally strain  
81 DSM 7029<sup>T</sup> was classified as a new species of the genus *Polyangium* (*Polyangiaceae*,  
82 *Myxococcales*), and named, although with a non-valid name, as “*Polyangium brachysporum*”  
83 based on phenotypic traits (Konishi et al., 1987). In 2007 the analysis of the 16S rRNA gene  
84 sequence revealed that strain DSM 7029 belonged to the order *Burkholderiales* (Schellenberg  
85 et al., 2007), which was supported by the analysis of its genome and further characterization  
86 (Tang et al., 2019, Tang et al., 2015).

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## 88 **8.2. Cell morphology:**

89 The cells of *Schlegelella thermodepolymerans* strain K14<sup>T</sup> are Gram-negative, non-spore  
90 forming, 0.5-0.6 µm wide and 1.0-2.8 µm long rods, motile by means of one polar flagellum  
91 (Elbanna et al., 2003). A similar cell morphology is observed in other *Schlegelella*  
92 *thermodepolymerans* members, strains DhA-71 and N2-680 (Elbanna et al., 2003, Manaia et

93 al., 2003), as well as in *Schlegelella aquatica* wcf1<sup>T</sup> and *Schlegelella brevitalea* DSM 7029<sup>T</sup>  
94 (0.4-0.5 and 0.4-0.6 µm in width, and 0.8-2.0 and 1.2-2.5 µm in length, respectively) (Chou et  
95 al., 2006, Tang et al., 2019). Intracellular poly-beta-hydroxybutyrate (PHB) granules have been  
96 reported in strains N2-680, wcf1<sup>T</sup> and DSM 7029<sup>T</sup> (Chou et al., 2006, Manaia et al., 2003, Tang  
97 et al., 2019).

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### 99 **8.3. Colonial and cultural characteristics:**

100 *Schlegelella thermodepolymerans* forms white and smooth convex colonies, with entire  
101 margins, either opaque (strain K14<sup>T</sup>) or translucent (strain DhA-71) (Elbanna et al., 2003, Yu  
102 and Mohn, 1999). After 36–48 h growth on Luria Bertani (LB) agar at 50 °C, *Schlegelella*  
103 *thermodepolymerans* N2-680 produces non-pigmented colonies, 1-2 mm in diameter (Manaia  
104 et al., 2003). *Schlegelella aquatica* wcf1<sup>T</sup> shows a similar colonial morphology, forming  
105 circular, convex, cream or white colonies with entire edges 1.0-1.5 mm in diameter on 10% LB  
106 agar after 48 h at 55 °C (Chou et al., 2006). After 48 h at 30 °C on CYCG (6.0 g L<sup>-1</sup> casitone,  
107 2.0 g L<sup>-1</sup> yeast extract, 1.4 g L<sup>-1</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.5% (v/v) glycerol, pH 7.0) agar medium,  
108 *Schlegelella brevitalea* DSM 7029<sup>T</sup> forms circular, convex, semi-transparent and greenish  
109 yellow colonies, with a smooth surface and a regular margin. It often produces green pigments  
110 on the same medium when grown over 3 days (Tang et al., 2019).

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### 112 **8.4. Nutrition and growth conditions:**

113 The type strain of *Schlegelella thermodepolymerans* (K14<sup>T</sup>) and strain N2-680 grow in mineral  
114 medium with single carbon sources such as gluconate, citrate and acetate, under aerobic  
115 conditions (Elbanna et al., 2003, Manaia et al., 2003, Tang et al., 2019). Vitamines improve  
116 the growth of strain K14<sup>T</sup> (Elbanna et al., 2003) whereas strain DhA-71 requires 300 mg L<sup>-1</sup>

117 Tryptic Soy Broth (TSB) when growing in mineral medium with single carbon sources, such  
118 as glycerol (Yu and Mohn, 1999).

119 The mineral medium MSM used for routine cultivation of *Schlegelella thermodepolymerans*  
120 contains per liter of distilled water: 9.0 g Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 1.5 g KH<sub>2</sub>PO<sub>4</sub>, 1.0 g NH<sub>4</sub>Cl, 0.2 g  
121 MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.2 mg FeNH<sub>4</sub>-Citrate, 20 mg CaCl<sub>2</sub>, 0.5 g NaHCO<sub>3</sub>, and 2 mL Hoagland  
122 solution (pH 6.8-7.0) (Schlegel et al., 1961). Whenever necessary, the mineral medium should  
123 be supplemented with 20 µg L<sup>-1</sup> biotin, 20 µg L<sup>-1</sup> folic acid, 60 µg L<sup>-1</sup> lipoic acid, 50 µg L<sup>-1</sup>  
124 thiamin, 50 µg L<sup>-1</sup> riboflavin, 50 µg L<sup>-1</sup> nicotinic acid, 100 µg L<sup>-1</sup> pyridoxal hydrochloride, 50  
125 µg L<sup>-1</sup> pantothenic acid, 50 µg L<sup>-1</sup> vitamin B12, 50 µg L<sup>-1</sup> p-aminobenzoic acid, and 200 µg L<sup>-1</sup>  
126 1 naphthoquinone (Elbanna et al., 2003). *Schlegelella thermodepolymerans* grows also well on  
127 complex media such as Tryptic Soy Agar, Nutrient Agar (NA) and LB agar, under aerobic  
128 conditions (Elbanna et al., 2003, Manaia et al., 2003). In contrast, *Schlegelella aquatica* strain  
129 wcf1<sup>T</sup> grows well in complex media with a reduced concentration of nutrients, such as 10%  
130 LB medium, whereas in TSB, NA and LB growth is comparatively poor (Chou et al., 2006).  
131 *Schlegelella brevitalea* strain DSM 7029<sup>T</sup> grows well in CYCG or Reasoner's 2A (R2A) media  
132 (Tang et al., 2019).

133 The temperature and pH range for growth of *Schlegelella thermodepolymerans* strain K14<sup>T</sup> is  
134 37-60 °C and 6-9, with optima at around 50 °C and pH 7 (Elbanna et al., 2003). Slight  
135 differences are found for strain DhA-71 which grows in the temperature range of 30-60 °C and  
136 pH 5-9 (Elbanna et al., 2003), and strain N2-680, which grows at 30-57 °C, pH 6-9, and NaCl  
137 up to 3% (w/v) (Manaia et al., 2003). *Schlegelella aquatica* wcf1<sup>T</sup> shares the cardinal  
138 temperature values with strain DhA-71 and has a narrower pH interval (pH 6-8) (Chou et al.,  
139 2006). *Schlegelella brevitalea* DSM 7029<sup>T</sup> has a narrower temperature interval (25-42 °C) and  
140 NaCl tolerance up to 0.2% (w/v), and a wider pH range (5-10), with optima at 30-37°C, pH 6-  
141 8 and without salt addition (Tang et al., 2019).

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143 **8.5. Metabolism:**

144 All *Schlegelella* spp. described at the moment of writing are aerobes, without visible growth  
145 under anaerobic conditions and are reported as being unable to ferment glucose (Chou et al.,  
146 2006, Elbanna et al., 2003, Manaia et al., 2003, Tang et al., 2019, Yu and Mohn, 1999). Nitrate  
147 reduction is a variable characteristic among *Schlegelella* strains. *Schlegelella*  
148 *thermodepolymerans* strain K14<sup>T</sup> reduces nitrate to nitrogen whereas strains DhA-71 and N2-  
149 680 do not, nor use it as an electron acceptor (Elbanna et al., 2003, Manaia et al., 2003, Yu and  
150 Mohn, 1999). *Schlegelella aquatica* wcf1<sup>T</sup> is reported as being able to reduce nitrate and  
151 *Schlegelella brevitalea* DSM 7029<sup>T</sup> to use nitrate as nitrogen source (Chou et al., 2006, Tang  
152 et al., 2019). The presence of photosynthetic pigments, N<sub>2</sub> fixation, ability to oxidize Mn<sup>2+</sup>, or  
153 to grow autotrophically in the presence of H<sub>2</sub> was tested only in *Schlegelella*  
154 *thermodepolymerans* strain N2-680 with negative results for all of these traits.

155 *Schlegelella thermodepolymerans* comprises chemo-organotrophs able to biodegrade polymers  
156 such as polyhydroxyalkanoates (PHAs) and polycaprolactones. In addition, they also degrade  
157 resin acids, a group of tricyclic diterpenes found particularly in conifers (Elbanna et al., 2003,  
158 Manaia et al., 2003, Yu and Mohn, 1999).

159 PHAs are thermoplastics and/or elastomers polyesters accumulated by bacteria from diverse  
160 taxonomic groups as intracellular carbon and energy storage compounds, when grown under  
161 nutrient stress (Lütke-Eversloh et al., 2004). Given their properties, PHAs or modified PHAs  
162 have been considered for various applications in industry, agriculture and medicine (Li et al.,  
163 2016, Lütke-Eversloh et al., 2004). *Schlegelella thermodepolymerans* K14<sup>T</sup> degrades both  
164 poly(3-hydroxybutyrate) (poly(3HB)), the most commonly PHA accumulated by bacteria, as  
165 well as the poly(3-hydroxybutyrate-co-3-mercaptopropionate) (poly(3HB-co-3MP))  
166 copolymer, a polythioester (PTE) accumulated by *Ralstonia eutropha* (*Cupriavidus necator*),

167 (Lütke-Eversloh et al., 2001a, Lütke-Eversloh et al., 2001b). In contrast, *Schlegelella*  
168 *thermodepolymerans* DhA-71 only degrades poly(3HB). The extracellular PHA  
169 depolymerase of *Schlegelella thermodepolymerans* K14<sup>T</sup>, which has a molecular mass of  
170 40.0±1.5 kDa, belongs to the serine hydrolase family, and has a specific activity with  
171 poly(3HB) and poly(3HB-co-3MP) of 25,000 and 9,500 units mg<sup>-1</sup>, respectively (Elbanna et  
172 al., 2004). It shows temperature and pH optima of 75 – 80 °C and 8, respectively, although it  
173 is active up to 90 °C and in the pH range of 6.0-10.5. This enzyme is specific for oxoester  
174 linkages and do not hydrolyse thioester bonds, which results in the accumulation of 3-  
175 mercaptopropionate containing oligomers (Elbanna et al., 2004).

176 *Schlegelella thermodepolymerans* strains K14<sup>T</sup> and DhA-71 are also able to degrade  
177 dehydroabietic acid, an abietane resin acid frequently present in paper mill effluents, which is  
178 toxic to fish (Elbanna et al., 2003, Yu and Mohn, 1999). *Schlegelella thermodepolymerans* N2-  
179 680 degrades polycaprolactone oxydiethylene ester, a poly-ε-caprolactone thermoplastic  
180 biodegradable homopolymer (Manaia et al., 2003).

181 Glidobactins are inhibitors of the human 20S proteasome, are cytotoxic to tumour cell lines,  
182 prolong the life span of mice inoculated with leukaemia cells and display a broad spectrum of  
183 antifungal activity (Bian et al., 2014, Oka et al., 1988, Schellenberg et al., 2007). These  
184 compounds were first isolated from *Schlegelella brevitalea* DSM 7029<sup>T</sup> that produces  
185 glidobactins A–C, acylated cyclic tripeptides differing in their side chain moieties (Oka et al.,  
186 1988). The gene cluster (*glbA-glbH*) encoding the putative enzymes involved in the glidobactin  
187 A biosynthesis in strain DSM 7029<sup>T</sup> includes a mixed non-ribosomal peptide/polyketide  
188 synthetase and has been successfully expressed in *Escherichia coli* (Bian et al., 2014,  
189 Schellenberg et al., 2007).

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## 191 **8.6. Chemotaxonomic characteristics:**



192 The fatty acid methyl esters profiles of *Schlegelella thermodepolymerans* strains K14<sup>T</sup> and  
193 DhA-71 at 50 °C on TSA medium are similar, with the predominance of C<sub>16:0</sub> (43.1%) and  
194 C<sub>17:0</sub> cyclo (32.6%), and lower percentages of C<sub>10:0</sub> 3-OH (3.3%), C<sub>12:0</sub> 3-OH (2.0%), and C<sub>18:1</sub>  
195  $\omega$ 7c (4.4%) (Elbanna et al., 2003). Strain N2-680 shows a similar profile when grown on LB  
196 agar at the same temperature, whereas at 30 °C unsaturated fatty acids increase and saturated  
197 and cyclo fatty acids decrease - C<sub>16:1</sub> (29.0%), C<sub>18:1</sub> (27.7%), C<sub>16:0</sub> (23.7%) and C<sub>17:0</sub> cyclo  
198 (6.5%) (Manaia et al., 2003). Although not cultivated simultaneously with the type strain of  
199 the type species (K14<sup>T</sup>), *Schlegelella aquatica* wcf1<sup>T</sup> grown at 50 °C on TSA medium shows a  
200 similar profile, with the predominance of C<sub>16:0</sub> (48.7%), followed by C<sub>16:1</sub>  $\omega$ 7c and/or C<sub>15:0</sub> iso  
201 2-OH (19.5%), and C<sub>17:0</sub> cyclo (16.5%) (Chou et al., 2006). According to Tang et al. (2019),  
202 the predominant fatty acids of *Schlegelella brevitalia* DSM 7029<sup>T</sup> are C<sub>16:1</sub>  $\omega$ 7c and/or C<sub>16:1</sub>  
203  $\omega$ 6c (41.6%), followed by C<sub>16:0</sub> (29.9%), C<sub>18:1</sub>  $\omega$ 7c (13.0%) and C<sub>10:0</sub> 3-OH (6.2%), probably  
204 when grown on CYCG agar medium at 30 °C.

205 The major respiratory quinone reported in *Schlegelella thermodepolymerans* N2-680 and  
206 *Schlegelella brevitalia* DSM 7029<sup>T</sup> is ubiquinone 8 (Manaia et al., 2003, Tang et al., 2019).

207 The major polar lipids reported for *Schlegelella thermodepolymerans* N2-680 are  
208 phosphatidylethanolamine and phosphatidylglycerol (Manaia et al., 2003). In *Schlegelella*  
209 *brevitalia* DSM 7029<sup>T</sup>, diphosphatidylglycerol, phosphatidylglycerol and  
210 phosphatidylethanolamine were reported as predominant, and two unidentified phospholipids  
211 and five unidentified lipids are minor components (Tang et al., 2019).

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## 213 **8.7. Genome:**

214 The draft genome of *Schlegelella thermodepolymerans* K14<sup>T</sup> (= DSM 15344<sup>T</sup>) is available  
215 under the DDBJ/EMBL/GenBank accession no. NZ\_QQAP000000000 (DOE Joint Genome  
216 Institute, unpublished) and NZ\_PSNY000000000 (Zhejiang Academy of Agricultural Sciences,

217 unpublished). Both sequences were obtained using Illumina HiSeq technology but with  
218 different assemblers (Velvet and SPAdes). The genome has a size of 3.83 Mb, with a total of  
219 3520-3531 candidate protein-coding genes and a G+C content of 70.3 mol%.

220 The draft genome of *Schlegelella thermodepolymerans* N2-680 (= DSM 15264) is available  
221 under the DDBJ/EMBL/GenBank accession no. NZ\_SLXF000000000 (DOE Joint Genome  
222 Institute, unpublished). It has a size of 4.03 Mb, with a total of 3744 candidate protein-coding  
223 genes and a G+C content of 70.2 mol%.

224 The complete genome of *Schlegelella brevitalea* strain DSM 7029<sup>T</sup> is available under the  
225 DDBJ/EMBL/GenBank accession no. CP011371 (Tang et al., 2015). The sequence was  
226 obtained using the Roche 454 GS FLX Titanium system. After a first assemblage with  
227 Newbler, the ContigScape was used to order contigs, and Sanger sequencing and the  
228 Phred/Phrap/Consed package were used to close the sequence. The consensus circular  
229 sequence has 6.48 Mb and contains 5557 genes, from which 3999 encode predicted proteins  
230 for which a biological function could be defined, and 1558 encode for hypothetical proteins  
231 (Tang et al., 2015).

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### 233 **8.8. Ecology:**

234 *Schlegelella* spp. have a ubiquitous distribution. Members of *Schlegelella thermodepolymerans*  
235 have been recovered mainly from human impacted environments. Strain K14<sup>T</sup> was isolated  
236 from activated sludge collected in Fayoum, Egypt, enriched in mineral medium with  
237 poly(3HB-co-3MP) as the single carbon and energy source and incubated at 55 °C (Elbanna et  
238 al., 2003). Strain DhA-71 was co-isolated with strain DhA-72 from a woody municipal  
239 compost sample collected in Richmond, British Columbia, Canada, enriched in mineral  
240 medium with dehydroabietic acid and isopimaric acid, at the same conditions (Yu and Mohn,  
241 1999). Four bacterial strains isolated from hot compost of a commercial composting plant in

242 Umweltschutz Nord, Ganderkesee, Germany, were affiliated to the genus *Schlegelella* since  
243 had a high 16S rRNA gene sequence similarity (98.1–99.8%) with strain K14<sup>T</sup> (Romen et al.,  
244 2004). Strain N2-680 was recovered from activated sludge surplus of a wastewater treatment  
245 plant in northern Portugal, which went through a mesophilic anaerobic digestion followed by  
246 a thermophilic aerobic digestion with a maximal temperature of 60 °C. A sample of the digested  
247 sludge was used as inoculum for enrichment in mineral medium with poly-ε-caprolactone  
248 thermoplastic at 50 °C (Manaia et al., 2003).

249 In contrast, *Schlegelella aquatica* wcf1<sup>T</sup> was recovered from a pristine thermophilic  
250 environment, a water sample collected in a hot spring in Guanzingling, southern Taiwan with  
251 pH around 8 and temperature ranging 60 to 65 °C (Chou et al., 2006). *Schlegelella brevitalea*  
252 DSM 7029<sup>T</sup> was isolated from a soil sample collected near the Parthenon in Athens, Greece,  
253 cultured in synthetic medium (Konishi et al., 1987, Tang et al., 2019).

254 The ability of *Schlegelella* to degrade dehydroabietic acid may also explain the high proportion  
255 of *Schlegelella*, together with *Chloroflexi*, and *Tepidimonas*, in thick and viscous deposits  
256 formed around the paper machines in papermaking plants, as assessed by screening of 16S  
257 rRNA gene libraries (Disnard et al., 2011). More surprisingly is the potential occurrence of  
258 *Schlegelella* DNA in nucleic acid extraction kit reagents, as suggested by Salter et al. (2014).

259 Organisms affiliated to *Schlegelella* have been also identified in the human gut microbiome,  
260 where a positive correlation with the age was observed (Scepanovic et al., 2019). The  
261 postpartum cervical microbiota of HIV-positive women displaying diverse cervical  
262 intraepithelial neoplasia includes *Schlegelella* spp., where despite the low abundance, were  
263 associated with cervical lesions (Curty et al., 2017). However, the role of *Schlegelella* spp. in  
264 the cervix is unknown (Curty et al., 2017).

265

266 **9. ENRICHMENT/ISOLATION PROCEDURES:**

267 *Schlegelella thermodepolymerans* K14<sup>T</sup> was isolated from an enrichment culture established  
268 from activated sludge (5 mL) in MSM medium (50 mL) with 0.15% poly(3HB-co-3MP) as  
269 sole carbon source (Elbanna et al., 2003). The culture was incubated at 55 °C for 1 week. Serial  
270 dilutions of the enrichment culture were spread on poly(3HB-co-3MP)-overlay MSM agar  
271 plates, where isolate K14<sup>T</sup> formed a clear zone (Elbanna et al., 2003). The poly(3HB-co-3MP)  
272 polymer was prepared according to the procedure described by Jendrossek et al. (1993).

273 To isolate *Schlegelella thermodepolymerans* DhA-71, an aliquot of two grams of compost was  
274 mixed with 20 mL of BR medium containing 200 mM of dehydroabietic acid (DhA) and 200  
275 mM isopimaric acid (IpA) in a sealed 250-mL flask. The culture incubated at 55 °C. The flask  
276 was opened and shaken twice per day. After two days, 0.4 mL of the culture was transferred to  
277 tightly capped 28-mL tubes containing 3.6 mL of BR medium and 100 mM of DhA and IpA.  
278 These cultures incubated horizontally without shaking at 55 °C. After every 48 h, 10% of the  
279 culture was replaced with the same volume of fresh BR medium. This semi-continuous  
280 enrichment process lasted three weeks, after which the culture was streaked on solid BR  
281 containing either DhA of high purity as the sole organic substrate and 1.5% purified agar. The  
282 plates were sealed in plastic bags and incubated at 55 °C. Individual colonies became visible  
283 after three to five days incubation and were transferred to 2.5-mL liquid BR medium containing  
284 DhA (Yu and Mohn, 1999).

285 *Schlegelella thermodepolymerans* N2-680 was isolated from an enrichment culture prepared  
286 with digested sludge (1 g) in mineral medium A (10 mL) supplemented with a pellet of poly-  
287  $\epsilon$ -caprolactone thermoplastic (oxepanone homopolymer, with a molecular mass of 80 000).  
288 Cultures were transferred weekly to fresh medium for 2 months, after which isolate N2-680  
289 was recovered by sub-culturing on LB agar (Manaiia et al., 2003).

290 To isolate *Schlegelella aquatica* wcf1<sup>T</sup>, a sample of water collected from a hot spring in  
291 southern Taiwan was diluted with sterile distilled water and plated on 10% LB agar plates. The

292 cultures incubated at 55°C. Strain wcf1<sup>T</sup> was purified by sub-cultivation on the same medium  
293 (Chou et al., 2006).

294

## 295 **10. MAINTENANCE PROCEDURES:**

296 *Schlegelella thermodepolymerans* can be maintained on complex solid media such as LB agar  
297 for short periods or in LB broth with 15% (v/v) glycerol at -80 °C (Manaia et al., 2003).

298 *Schlegelella aquatica* wcf1<sup>T</sup> can be maintained on 10% LB medium (Chou et al., 2006). The  
299 type strains of the *Schlegelella* species are supplied as freeze dried cultures by different culture  
300 collections (e.g., DSMZ, ATCC, BCCM/LMG).

301

## 302 **11. DIFFERENTIATION OF THE GENUS SCHLEGELELLA FROM OTHER**

### 303 **GENERA:**

304 The closest related genera to *Schlegelella* are *Caldimonas*, *Zhizhongheella*, *Aquabacterium*  
305 (see gbm00953), *Calidifontimicrobium*, *Piscinibacter*, *Aquincola*, and *Methylibium*.

306 Differentiating characteristics between the *Schlegelella* strains and those of related genera are  
307 given in Table 1 (detailed information for all the strains available in supplementary table S1).

308 The relatively close taxonomic relatedness between genera within the family  
309 *Comamonadaceae* (Willems, 2014) together with the metabolic versatility of the organisms

310 affiliated to these taxa make it difficult to differentiate *Schlegelella* based on chemotaxonomic  
311 or phenotypic traits. Nevertheless, based on the organisms affiliated so far to these genera,

312 *Schlegelella* differs from *Zhizhongheella* and *Methylibium* on the inability to grow below 15  
313 °C or under anaerobic conditions. This last trait also distinguishes *Schlegelella* from

314 *Calidifontimicrobium*, which organisms are capable of anaerobic growth.

315

316 <Table 1 near here>

317

318

319 **12. TAXONOMIC COMMENTS:**

320 Based on the 16S rRNA gene sequence analyses, *Schlegelella* is a member of the family  
321 *Comamonadaceae* (see fbm00182). *Calidifontimicrobium sediminis*, *Aquabacterium*  
322 *tepidiphilum*, “*Caldimonas meghalayensis*”, *C. manganoxidans*, *C. taiwanensis*, “*C.*  
323 *hydrothermale*”, *Zhizhongheella caldifontis* and *Piscinibacter caeni* are the closest neighbour  
324 species of *Schlegelella thermodepolymerans* K14<sup>T</sup>. The type strains of these species share 96.8-  
325 96.1% 16S rRNA gene pairwise sequence similarity with strain K14<sup>T</sup>. Whereas the type strains  
326 of *C. manganoxidans*, *C. taiwanensis*, “*C. hydrothermale*”, “*C. meghalayensis*”, *Z. caldifontis*,  
327 *A. tepidiphilum* and *Calidifontimicrobium sediminis* share 96.6-96.0% 16S rRNA gene  
328 pairwise sequence similarity with *Schlegelella aquatica* wcf1<sup>T</sup>. Both strains K14<sup>T</sup> and wcf1<sup>T</sup>  
329 share lower 16S rRNA gene pairwise sequence similarity values (<95.9%) with the type strains  
330 of different species of the genera *Ideonella*, *Aquincola*, *Rubrivivax*, *Leptothrix*,  
331 *Azohydromonas*, *Methylibium* and *Tepidimonas*.

332 *Schlegelella thermodepolymerans* K14<sup>T</sup> and *Schlegelella aquatica* wcf1<sup>T</sup> share a high value of  
333 16S rRNA pairwise gene sequence similarity (98.0%). Nevertheless, these organisms are  
334 differentiated by the low DNA–DNA relatedness value (45.0%), along with differentiating  
335 physiological and biochemical traits (Chou et al., 2006). Specifically, *Schlegelella aquatica*  
336 wcf1<sup>T</sup> has a higher percentage of C<sub>16:1ω7c</sub> and/or C<sub>15:0</sub> iso 2-OH than *Schlegelella*  
337 *thermodepolymerans*. In addition, in contrast with the *Schlegelella thermodepolymerans*  
338 strains, *Schlegelella aquatica* wcf1<sup>T</sup> is catalase- and cystine arylamidase negative, α-

339 glucosidase positive, and it is unable to assimilate citrate as sole carbon source (Chou et al.,  
340 2006).

341 Surprisingly, *Schlegelella brevitalea* DSM 7029<sup>T</sup> shares lower 16S rRNA gene pairwise  
342 sequence similarity with strains K14<sup>T</sup> and wcf1<sup>T</sup> (96.8% and 95.7%, respectively) than with  
343 the type strains of *Aquincola tertiaricarbonis*, *Calidifontimicrobium sediminis*, *Piscinibacter*  
344 *caeni*, *Methylibium petroleiphilum*, *Piscinibacter defluvii* and *Aquabacterium tepidiphilum*  
345 (97.2-97.0%). Slightly lower values (96.8-96.0%) are shared between strain DSM 7029<sup>T</sup> and  
346 the type strains of numerous species, such as *Rivibacter subsaxonicus*, *Zhizhongheella*  
347 *caldifontis*, *Ideonella azotifigens*, *Rhizobacter bergeniae*, *Rubrivivax benzoatilyticus*,  
348 *Azohydromonas australica* and *Leptothrix discophora*. Among the closest neighbours, strain  
349 *Schlegelella brevitalea* DSM 7029<sup>T</sup> shares high average nucleotide- (ANI) and amino acid  
350 identity (AAI) values with *Schlegelella thermodepolymerans* K14<sup>T</sup> (78.31% and 73.31%,  
351 respectively) (Tang et al., 2019), which are, respectively, below and above the proposed species  
352 (95%) (Richter et al., 2016) and genus (65%) boundaries (Konstantinidis et al., 2017). In  
353 addition, the value of percentage of conserved protein (POCP) between *Schlegelella brevitalea*  
354 DSM 7029<sup>T</sup> and *Schlegelella thermodepolymerans* K14<sup>T</sup> is 59.23% (Tang et al., 2019), which  
355 is higher than the proposed genus boundary (50 %) (Qin et al., 2014).

356 However, *Schlegelella thermodepolymerans* K14<sup>T</sup> shares AAI and POCP values above or close  
357 to the proposed genus thresholds with the type strains of species of other genera, such as *Z.*  
358 *caldifontis* YIM 78140<sup>T</sup> (73.01% and 55.18%, respectively) and *C. manganoxidans* HS<sup>T</sup>  
359 (72.57% and 54.08%, respectively). Moreover, the AAI and POCP values shared between *Z.*  
360 *caldifontis* YIM 78140<sup>T</sup> and *C. manganoxidans* HS<sup>T</sup> (84.29% and 80.7%, respectively), or  
361 between each of these strains with *S. thermodepolymerans* K14<sup>T</sup> (73.00% and 68.57%, and  
362 72.58% and 65.41%, respectively), are also higher than the proposed genus thresholds (Tang

363 et al., 2019), indicating close taxonomic relatedness between genera within the  
364 *Comamonadaceae* family (Willems, 2014).

365 In spite the fact that the AAI and POCP analyses do not exclude *Schlegelella brevitalea* DSM  
366 7029<sup>T</sup> from the genus *Schlegelella*, the 16S rRNA gene based phylogenetic inference suggests  
367 that it might be better placed in another group (Figure 1). Moreover, also the relationship of  
368 members of the genus *Schlegelella* with neighbour genera, in particular the species *C.*  
369 *manganoxidans* and *Z. caldifontis*, may need to be reviewed based on an integrated analysis of  
370 the *Comamonadaceae* family.

371

372 <Figure 1 near here>

373

### 374 **13. LIST OF SPECIES OF THE GENUS *SCHLEGELELLA*:**

375 1. *Schlegelella thermodepolymerans* Elbanna, Lütke-Eversloh, Van Trappen, Mergaert,  
376 Swings and Steinbüchel 2003, 1167<sup>VP</sup>

377 ther'mo.de.po.ly'me.rans. Gr. n. *therme* heat; N.L. v. *depolymerare* to depolymerize; N.L. part.  
378 adj. *thermodepolymerans* depolymerizing in the heat, referring to the ability to degrade poly(3-  
379 hydroxybutyrate) at high temperatures.

380

381 In addition to the characteristics given in the genus description, strains are positive for the  
382 utilization of gluconate and citrate as single carbon sources. Catalase and oxidase positive.  
383 Susceptible to nalidixic acid, and resistant to penicillin G and ampicillin.

384

385 The DNA G+C content (mol %) is 70.0-70.1 (HPLC) or 70.2-70.3 (genome analysis,  
386 GenBank).



387 Type strain: K14 (=LMG 21644= DSM 15344)

388 GenBank accession number (16S rRNA): AY152824

389 GenBank accession number (genome): NZ\_QQAP000000000 and NZ\_PSNY000000000

390

391 2. *Schlegelella aquatica* Chou, Sheu, Sheu, Wang and Chen 2006, 2796<sup>VP</sup>

392 a.qua'ti.ca. L. fem. adj. *aquatica* living in water.

393

394 In addition to the characteristics given in the genus description, the type strain shows the  
395 following properties: tests positive for gelatin- and aesculin hydrolysis, C4 esterase, C8  
396 esterase lipase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and  $\alpha$ -glucosidase,  
397 and weakly positive for oxidase activity. Catalase negative. Assimilates glucose, maltose,  
398 gluconate, adipate, lactate, acetate, succinate, pyruvate and phenyl acetate. Susceptible to  
399 rifampicin, streptomycin, penicillin G and ampicillin.

400

401 The DNA G+C content (mol %) is 69.2 (HPLC).

402 Type strain: wcf1 (=BCRC 17557=LMG 23380)

403 GenBank accession number (16S rRNA): DQ417336

404

405 3. *Schlegelella brevitalea* Tang, Yu, Liang, Zhang, Bian, Zhi and Ding 2019, 2882<sup>VP</sup>

406 bre.vi.ta'le.a. L. adj. *brevis* short; L. fem. n. *talea* a rod; N.L. fem. n. *brevitalea* a short rod.

407

408 In addition to the characteristics given in the genus description, the type strain tests positive for  
409 catalase and negative for oxidase. It uses sucrose, dextrin, starch and glycerol, and weakly uses  
410 D-mannitol and D-inositol, as carbon source. In addition, it uses L-asparagine, L-proline, L-

411 tyrosine, L-glutamine, DL-aspartic acid, ammonium sulfate and sodium nitrate, and weakly  
412 use L-serine, L-histidine, L-isoleucine, L-valine, L-methionine and L-cysteine, as nitrogen  
413 sources. The whole-cell hydrolysates contain glucose, rhamnose and lysine, alanine and  
414 glycine.

415

416 The DNA G+C content (mol %) is 67.5 (genome analysis, GenBank).

417 Type strain: DSM 7029 (=K481-B101 = ATCC 53080)

418 GenBank accession number (genome): NZ\_CP011371.1

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420

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583

584 Table 1. Differentiating characteristics between *Schlegelella* and the closest genera.

585

	<i>Schlegelella</i>	<i>Aquabacterium</i>	<i>Aquicola</i>	<i>Calidifontimicrobiu</i>	<i>Caldimonas</i>	<i>Methylibium</i>	<i>Piscinibacter</i>	<i>Zhizhongheella</i>
Trait	(n=5)	(n=8)	(n=5)	<i>m</i> (n=2)	(n=4)	(n=1)	(n=3)	(n=1)
Motility	+	v	+	+	+	+	v	+
Poly-β-hydroxybutyrate accumulation	+	v	- (3 strains)	ND	+	+	+ (1 strain)	ND
Optimal growth ≥ 45 °C	v	v	-	+	+	-	-	+
Growth below 15 °C	-	v	v	-	-	+	v	+
Anaerobic growth	-	v	-	+	-	+	v	+
Catalase	v	v	+	+	+	-	v	+
Oxidase	v	+	+	+	v	+	+	+
DNA G+C content (mol%)	69.2-70.1	66-70.7	70.1-70.7	71.8 (1 strain)	65.9-68.3	69	66.2-70.1	70.8

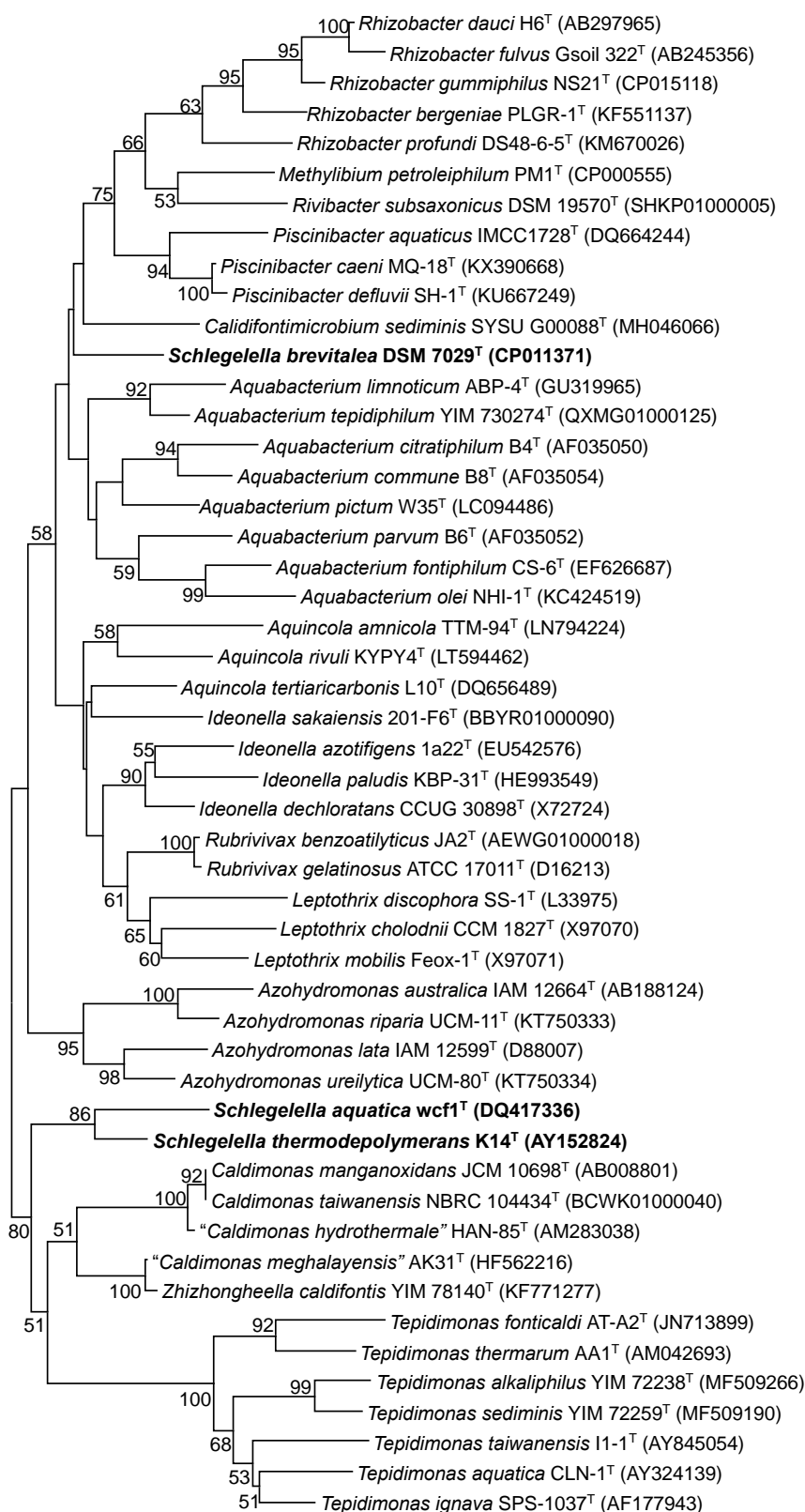
586

587 +, positive; -, negative; v, variable among strains.

588 *Schlegelella* include *S. thermodepolymerans* K14<sup>T</sup>, *S. thermodepolymerans* DhA-71, *S. thermodepolymerans* N2-680, *S. aquatica* wcf1<sup>T</sup> and *S. brevitalea* DSM 7029<sup>T</sup> (Chou et al., 2006, Elbanna et al., 2003, Manaia et al., 2003, Tang et al., 2019)590 *Aquabacterium* include *Aquabacterium citratiphilum* B4<sup>T</sup>, *Aquabacterium commune* B8<sup>T</sup>, *Aquabacterium fontiphilum* CS-6<sup>T</sup>, *Aquabacterium limnoticum* ABP-4<sup>T</sup>,591 *Aquabacterium olei* NHI-1<sup>T</sup>, *Aquabacterium parvum* B6<sup>T</sup>, *Aquabacterium tepidiphilum* YIM 730274<sup>T</sup>, and *Aquabacterium pictum* W35<sup>T</sup> (Chen et al., 2012, Hirose et al., 2020, Kalmbach et al., 1999, Khan et al., 2019, Lin et al., 2009, Pham et al., 2015).593 *Aquicola* include *A. tertiaricarbonis* L10<sup>T</sup>, *A. tertiaricarbonis* L108 and *A. tertiaricarbonis* CIP I-2052, *A. amnicola* TTM-94<sup>T</sup>, and *A. rivuli* KYPY4<sup>T</sup> (Chen et al., 2018b, Lechner et al., 2007, Sheu et al., 2019).595 *Calidifontimicrobium* include *Calidifontimicrobium sediminis* SYSU G00088<sup>T</sup> and *Calidifontimicrobium sediminis* YIM 73032 (Ding et al., 2019).596 *Caldimonas* include “*Caldimonas hydrothermale*” HAN-85<sup>T</sup>, *Caldimonas manganoxidans* HS<sup>T</sup>, “*Caldimonas meghalayensis*” AK31<sup>T</sup>, and *Caldimonas taiwanensis* On1<sup>T</sup> (Bouraoui et al., 2010, Chen et al., 2005, Rakshak et al., 2013, Takeda et al., 2002).598 *Methylibium* include *Methylibium petroleiphilum* PM1<sup>T</sup> (Nakatsu et al., 2006).599 *Piscinibacter* include *Piscinibacter caeni* MQ-18<sup>T</sup>, *Piscinibacter defluvii* SH-1<sup>T</sup> and *Piscinibacter aquaticus* IMCC1728<sup>T</sup> (Chen et al., 2018a, Cho et al., 2016, Song and Cho, 2007, Stackebrandt et al., 2009).601 *Zhizhongheella* include *Zhizhongheella caldifontis* YIM 78140<sup>T</sup> (Dong et al., 2014).

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0.0050

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606 Figure 1. Dendrogram based on 16S rRNA gene sequences, showing the position of the  
607 *Schlegelella* species in relation to the closest phylogenetic genera. The dendrogram was  
608 generated by the Neighbor-Joining method. Bootstrap values, generated from 1000 re-  
609 samplings, are indicated at branch points. Bar, 1 substitution per 200 nucleotide positions.

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