Chryseolinea serpens gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from soil

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An aerobic chemoheterotrophic gliding bacterium, designated RYG^T, was isolated from a soil in Germany. Cells were Gram-stain-negative, thin rods (0.4–0.6 μm in width and 2.0–5.5 μm in length). Cells multiplied by normal cell division and no resting stages were observed. Colonies were yellow and displayed swarming edges. Gliding motility was observed in wet mounts. Strain RYG^T grew at pH 5.6–7.7 (optimum pH 6.6–7.0), at 13–37 °C (optimum 25–30 °C) and with 0–1.0 % NaCl (optimum 0–0.1 %). The isolate was incapable of atmospheric nitrogen fixation and grew on most mono- and disaccharides as well as a few polysaccharides and organic acids. The predominant menaquinone was MK-7, the major cellular fatty acids were $C_{16:1}\omega5c$ and iso-$C_{15:0}$ and the major intact polar lipids were composed of phosphatidylethanolamine derivatives and two unknown series. The DNA G+C content was 49.9 mol%. Based on 16S rRNA gene sequence analysis, the isolate belonged to the phylum *Bacteroidetes*, class *Cytophagia*, order *Cytophagales*, but was only distantly related to any cultured bacteria. The closest relatives were *Ohtaekwangia koreensis* 3B-2^T and *Ohtaekwangia kribbensis* 10AO^T (both 93 % 16S rRNA gene sequence similarity). We propose a novel genus and species, *Chryseolinea serpens* gen. nov., sp. nov.. Strain RYG^T (=DSM 24574^T=ATCC BAA-2075^T) is the type strain.

Members of the phylum *Bacteroidetes* are abundant in soil environments, making up on average 5 % (range 0–18 %) of the 16S rRNA gene clones detected by cultivation-independent methods in soils (Janssen, 2006). This places the phylum as the fifth-most detected in soil habitats. There are over 100 genera of *Bacteroidetes* now described, but this represents only a small fraction of the extant diversity of the group.

Strain RYG^T was isolated from a soil sample collected in March 2003 from sediment at the bottom of a small ephemeral brook in a forest near Marburg, Germany. The isolate was obtained as a contaminant in an enrichment culture for a methanotrophic bacterium, *Methylocapsa aurea* (Dunfield *et al.*, 2010). In brief, plates of diluted nitrate mineral salts medium at pH 5.8 (DNMS; Dunfield *et al.*, 2003) were inoculated with soil and incubated for 2 months at 25 °C in a closed glass desiccator containing a headspace of 20 % methane and 5 % CO_2 (v/v) in air. During this time, yellowish colonies of irregular shape developed on the plates. These were picked and, after successive restreaking, a simple co-culture was obtained that contained a proteobacterial methanotroph (*Methylocapsa aurea* KYG^T) and a heterotrophic bacterium (strain RYG^T). Strain RYG^T was easily isolated from the mixed culture by streaking onto R2A agar (l^{-1} H_2O: 0.5 g yeast extract, 0.5 g protease peptone, 0.5 g casein hydrolysate, 0.5 g glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4, 0.05 g $MgSO_4.7H_2O$, 12.0 g agar; pH 7.2±0.2), where the methanotroph was incapable of growth. Once obtained in pure culture, strain RYG^T was maintained by subcultivation at 1-month intervals on R2A agar and by storage at −80 °C in 5 % DMSO. *Ohtaekwangia koreensis* KCTC 23018^T and *Ohtaekwangia kribbensis* KCTC 23019^T (Yoon *et al.*, 2011), were obtained from the Korean Collection for Type Culture, grown under the same culture conditions and used as reference strains for most tests.

Batch cultures in R2A broth displayed yellow turbidity. On R2A agar, strain RYG^T formed pinpoint (<1 mm diameter

Abbreviation: IPL, intact polar lipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RYG^T is FR774778.

A supplementary table is available with the online version of this paper.

after 1 week), deep-yellow colonies, with a raised circular central section surrounded by a flat ring typical of swarming motility. On dilute media (20- or 10 %-strength R2A agar), the thin, broad swarming edges were more evident (Fig. 1a). Wet mounts made from colonies taken from dilute media showed clear gliding motility along the glass surface. Morphological observations and cell size measurements were made using an Olympus BX51 microscope equipped with Image Pro Express 6.0 software. Cells of strain RYG^T were straight to slightly curved rods, 0.4–0.6 μm in width and 2.0–5.5 μm in length (Fig. 1b–d). Cells stained Gram-negatively and occurred singly or in irregularly shaped aggregates. Catalase activity was evaluated by production of oxygen bubbles in a 3 % (v/v) aqueous hydrogen peroxide solution and oxidase activity was tested by oxidation of 1 % (w/v) dimethyl-*p*-phenylenediamine. Catalase and oxidase activities were positive. No spores were observed. The production of flexirubin-type pigments was tested by flooding a mass of cells with 20 % KOH and observing changes in colony colour from yellow to red or brown (Bernardet & Bowman, 2006; Kim *et al.*, 2011). Flexirubin-type pigments were produced.

Samples for TEM were prepared by fixation in 2.5 % glutaraldehyde/0.2 % sodium cacodylate buffer overnight at room temperature, three washings in 0.2 M sodium cacodylate buffer, post-fix staining in 2 % osmium tetroxide/0.2 M sodium cacodylate buffer for 1 h, two washings in 0.1 M sodium cacodylate buffer, dehydration through a graded alcohol series (30, 50, 70, 85, 95, 100 % ethanol), rinsing with propylene oxide and embedding in epoxy resin (Fluka 45359 Epoxy Embedding Kit; Sigma Aldrich). Ultrathin sections were cut with a diamond knife on an ultramicrotome (Ultracut E; Reichert-Jung) and collected on electron microscopy grids. Sections were strained with 2 % aqueous uranyl acetate and Reynold's lead citrate prior to viewing on a Hitachi H7650 transmission electron microscope at 80 kV at the University of Calgary. Images were acquired with an AMT16000 digital camera (Advanced Microscopy Techniques) mounted on the microscope. Light (less dense) areas observed within the cells may be a storage compound such as polyhydroxybutyrate, polyglucose or polyphosphate (Fig. 1c).

To determine the maximum growth rate, 50 ml R2A broth in duplicate 100 ml serum vials was inoculated with broth-grown culture, which was checked for purity by plating, and incubated for 7 days at 25 °C on a rotary shaker at 200 r.p.m. Growth was monitored by nephelometry at 600 nm using a spectrophotometer (Carl Zeiss). The maximum OD_{600} in R2A broth (pH 7.25, 25 °C) was 0.4 and the exponential growth rate constant at room temperature was 1.1 h^{-1} (doubling time of 38 min).

Other growth tests of strain RYG^T, except where otherwise noted, were performed in R2A broth (50 ml in 100 ml serum vials) at ambient temperature (20–25 °C). Growth at various temperatures, pH and NaCl concentrations were tested and scored as positive or negative in comparison to an uninoculated control. To determine the temperature range, duplicate vials were incubated at 4, 13, 20, 24, 30, 37 and 45 °C. Positive growth was observed at 13–37 °C after 14 days. Growth at 37 °C was extremely weak, forming small aggregates but no diffuse turbidity, which suggested that this was very close to the upper limit. Based on the maximum observed growth rate (determined by testing OD_{600} at 24 h intervals), the optimum temperature was estimated to be 25–30 °C. Conditions for growth in R2A medium were also tested at pH 3.1, 4.1, 4.6, 5.0, 5.6, 5.9, 6.6, 7.0, 7.4, 7.7 and 8.8 and with 0.1 and 0.5–5.0 % (w/v) NaCl (at intervals of 0.5 %). Growth occurred at pH 5.6–7.7 (optimum pH 6.6–7.0) and with 0–1.0 % NaCl (optimum 0–0.1 %).

Growth on different nitrogen sources was tested in DNMS medium containing 0.05 % glucose and 0.05 % $NaNO_3$ (w/v), replacing the $NaNO_3$ with 0.05 % NH_4Cl, Casamino

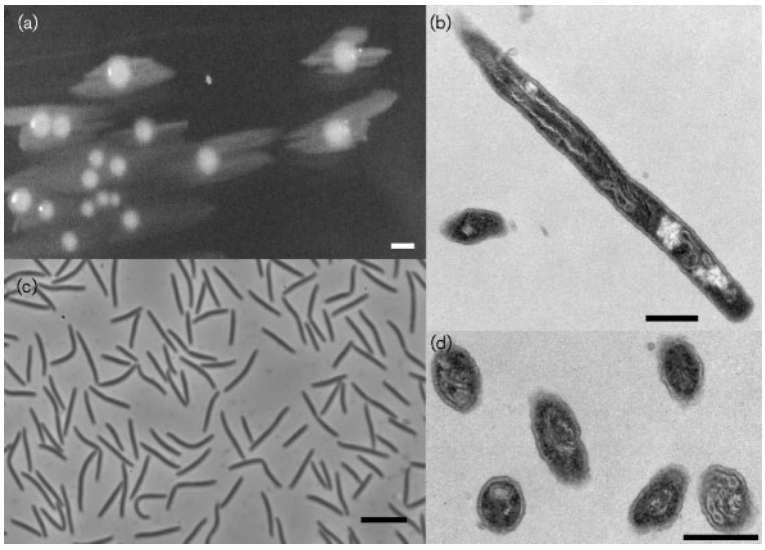

Fig. 1. Morphology of strain RYG^T. (a) Photograph of 5-day-old colonies grown on 0.1×strength R2A agar. (b) Phase-contrast micrograph of cells grown in R2A broth for 5 days. (c, d) Electron micrographs of ultrathin sections of methane-grown cells. Bars, 1 mm (a), 5 μm (b) and 500 nm (c, d).

acids or no nitrogen source. Growth was observed with $NaNO_3$, NH_4Cl and Casamino acids as the sole N source, but no growth was observed in N-free medium, even after an extended incubation of more than 5 months. PCR targeting *nifH* using primers considered universal to the gene (Zehr & McReynolds, 1989; Jurelevicius, *et al.*, 2010) failed to produce any amplification products, supporting the conclusion that strain RYG^T was incapable of nitrogen fixation.

Aerobic utilization of various substrates was determined using Biolog GN2 MicroPlates (Table S1, available in IJSEM Online). Test results were positive for most mono- and disaccharides tested, but was negative for all alcohols except for some sugar alcohols. A few organic acids and amino acids also showed positive reactions. In addition, xylan, pectin, starch, alginic acid and CM-cellulose were supplemented to DNMS liquid medium (each at a concentration of 0.05 %, w/v) to determine growth of RYG^T on some complex carbohydrates. Growth was positive on xylan, pectin and starch, but not on alginic acid or CM-cellulose.

Anaerobic growth was tested in R2A broth and in DNMS supplemented with glucose at 0.05 % (w/v). Duplicate vials were capped gas tight with butyl rubber stoppers and evacuated four times (5 min) and refilled each time with N_2. Fermentation or denitrification should have been possible under these conditions. No growth was observed even after 4 months of incubation. To test for capnophilic growth, anaerobic cultures in R2A broth were grown under different headspace conditions (100 % N_2, 100 % CO_2 and 90 % N_2 + 10 % CO_2) at 25 °C and 200 r.p.m. for 1 month. No growth was observed in any case.

Enzyme activities were tested using the API ZYM system (bioMérieux). Strain RYG^T was positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-glucosidase. Negative tests were obtained for trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase and α-fucosidase. The presence of enzyme activities of lipase (C14), cystine arylamidase, α-galactosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase were uncertain.

Fatty acids were released by acid hydrolysis (1 M HCl in methanol) from harvested batch cultures grown in R2A broth for 10 days and analysed by GC/MS, as described by Kulichevskaya *et al.* (2012). The position of double bonds were determined by dimethyl disulfide derivatization (Vincent *et al.*, 1987). The results are shown in Table 1, alongside those of members of the genus *Ohtaekwangia* obtained in this study. Saturated or monounsaturated C_{15}–C_{17} fatty acids predominated, with $C_{16:1}\omega5c$ (29.4 %) and iso-$C_{15:0}$ (17.4 %) as the most abundant fatty acids. About 25 % of the fatty acids comprised hydroxyl fatty acids. Analysis of the intact polar lipids (IPLs) was performed by Bligh/Dyer extraction and analysis by LC/MS as described by Kulichevskaya *et al.* (2012). The major IPLs were phosphatidylethanolamine derivatives and two unknown series (Table 2).

Table 1. Cellular fatty acid content of strain RYG^T and its closest phylogenetic neighbours

Strains: 1, *Chryseolinea serpens* gen. nov., sp. nov. RYG^T; 2, *Ohtaekwangia koreensis* KCTC 23018^T; 3, *Ohtaekwangia kribbensis* KCTC 23019^T. All data were obtained in this study using cells grown on R2A agar at 30 °C for 5 days (stationary phase). Major fatty acids (>5 %) are indicated in bold. Fatty acids comprising <1.0 % of the total in all strains are not shown. tr, Trace (<1.0 %); −, not detected.

Fatty acid (%)	1	2	3
Saturated			
$C_{15:0}$	**5.0**	1.5	2.7
$C_{16:0}$	**6.9**	1.1	1.8
Unsaturated			
$C_{15:1}\omega4$	2.9	**5.1**	**5.9**
$C_{16:1}\omega5$	**29.4**	**18.3**	**19.5**
$C_{16:1}\omega12$	3.3	2.0	1.7
Branched			
iso-$C_{13:0}$	**5.0**	4.4	3.0
iso-$C_{14:0}$	tr	tr	1.6
iso-$C_{15:0}$	**17.4**	**37.1**	**26.2**
iso-$C_{15:1}\omega11$	−	1.6	−
iso-$C_{16:0}$	tr	1.5	3.6
iso-$C_{17:1}\omega6+\omega13$	−	1.1	tr
Hydroxy			
$C_{14:0}$ 3-OH	1.8	tr	tr
$C_{15:0}$ 2-OH	1.5	tr	tr
$C_{16:0}$ 2-OH	1.0	−	tr
$C_{16:0}$ 3-OH	3.0	tr	1.4
$C_{16:1}\omega7$ 2-OH	2.0	−	1.1
iso-$C_{15:0}$ 2-OH	**8.6**	**8.3**	**10.5**
iso-$C_{15:0}$ 3-OH	**5.4**	**6.3**	**6.2**
iso-$C_{17:0}$ 3-OH	2.1	**5.2**	4.3
iso-$C_{16:0}$ 2-OH	tr	tr	1.9

The respiratory quinones of strain RYG^T were extracted and purified by the method of Hiraishi & Kato (1999) and were separated by TLC on silica gel 60 F_{254}, 20 HPTLC aluminium sheets (5 × 7.5 cm; Merck) using *n*-hexane/diethyl ether (97 : 3, v/v) as the developing solution, with MK-6 from *Flavobacterium hercynium* WB 4.2-33^T (Cousin *et al.*, 2007) and MK-7 from *Geobacillus toebii* SK-1^T (Sung *et al.*, 2002) as references. Strain RYG contained MK-7 as the major menaquinone, as for all members of the class *Cytophagia*.

DNA was extracted from a culture using a FastDNA kit (QBiogene). The 16S rRNA gene was amplified in a reaction mixture (50 µl) containing FailSafe PCR 2 × Premix F (Epicentre Biotechnologies), 1 U *Taq* DNA polymerase, 0.5 µM of each of the universal primers 9f and 1492b and 1 µl DNA extract. The thermal cycling program was an initial denaturing step at 94 °C for 5 min; then 33 cycles at 94 °C for 45 s, 48 °C for 1 min and 72 °C for 2 min, and a final elongation step at 72 °C for 5 min. Amplification

Table 2. Relative abundances and compositions of major intact polar lipids of strain RYG^T and its closest phylogenetic neighbours

Strains: 1, *Chryseolinea serpens* gen. nov., sp. nov. RYG^T; 2, *Ohtaekwangia koreensis* KCTC 23018^T; 3, *Ohtaekwangia kribbensis* KCTC 23019^T. Data from this study. IPLs are listed in order of elution. Abundances are relative to the major peak in the LC/MS base peak chromatogram. Note that the mass spectral response factors for different IPL groups can be quite different. The predominant fatty acid composition is reported, where possible, as the total number of carbon atoms of the acyl moieties and the number of double bonds. PE, Phosphatidylethanolamine; +++, 100 % of base peak; ++, 50–100 % of base peak; +, 20–50 % of base peak; −, <20 % of base peak.

IPL	1	2	3
PE	++ (32:1, 32:1, 31:2, 31:1)	+ (31:1, 32:2, 31:2, 30:0)	+ (31:1, 32:1, 32:2, 31:2)
Unknown*	++	−	−
Unknown†	+++	+++	+++

*This IPL contains a PE head group (loss of *m/z* 141) and contains two nitrogen atoms (MH^+ is odd; i.e. major ions at *m/z* 635.6, 647.6 and 649.6). It may be a sphingolipid IPL containing a PE group.
†This IPL seems to be an ornithine derivative since *m/z* 115 is always observed as a fragment ion. The MH^+ is, however, even (major ions at *m/z* 668.7 and 682.7, and corresponding Na adducts), indicating one or three nitrogen atoms and not two (as in case of ornithine IPLs).

products were purified using a QIAquick PCR purification kit (Qiagen) and sequenced on an Applied Biosystems 3730XL 96 capillary sequencer. A nearly complete sequence of 1472 bases was obtained. Phylogenetic analysis was carried out using ARB (Ludwig *et al.*, 2004).

Comparative sequence analysis of the 16S rRNA gene showed that strain RYG^T belonged to the phylum *Bacteroidetes* (Fig. 2), but had no close relatives among described species. Strain RYG^T belonged to a large group of sequences using cultivation-independent methods from a variety of environments, primarily soils and sediments (data not shown). Based on a BLAST search and pairwise alignment in ARB, the closest described phylogenetic relatives were *O. koreensis* 3B-2^T (93.0 % 16S rRNA gene sequence similarity) and *O. kribbensis* 10AO^T (92.7 %) (Yoon *et al.*, 2011). Strain RYG^T, the two members of the genus *Ohtaekwangia* and many environmental sequences formed a deeply rooted lineage with high bootstrap support. This group represents an as-yet unnamed taxonomic family or order. 16S rRNA gene sequence similarity with other cultured members of the phylum *Bacteroidetes* was <88 %, the next closest described relative being *Flexithrix dorotheae* ATCC 23163^T (GenBank accession number AB078077) with 87.6% 16S rRNA gene sequence similarity (Lewin, 1970). A taxonomically undescribed isolate from a freshwater sediment in Japan, strain TG141 (Tamaki *et al.*, 2009), showed 92 % 16S rRNA gene sequence similarity with strain RYG^T and belonged to the same phylogenetic cluster (data not shown). Interestingly, strain TG141 does not form visible colonies on agar like strain RYG^T does; however, it can grow when gellan gum is used as a gelling agent (Tamaki *et al.*, 2009).

For the determination of DNA G+C content of strain RYG^T, a thermal denaturation fluorimetric method (Xu *et al.*, 2000; Gonzalez & Saiz-Jimenez, 2002, 2004) using SYBR Green and a real-time PCR thermocycler (Rotor-Gene Q; Qiagen) was applied. Genomic DNA from *Flavobacterium johnsoniae* UW101^T (G+C content 34.1 mol%; http://genome.jgi-psf.org/flajo/flajo.home.html), *Bacillus subtilis* 168 (G+C content 43.5 mol%; Kunst *et al.*, 1997) and *Beijerinckia indica* subsp. *indica* ATCC 9039^T (G+C content 57.0 mol%; Tamas *et al.*, 2010), which have had their genomes completely sequenced, were used as the calibration references. Thermal denaturation was performed with approximately 2.5 μg DNA from the isolate and the calibration references. Thermal conditions consisted of a ramp from 55–99 °C at 0.5 °C min^{-1}. Fluorescent DNA melting curves were generated in triplicate. The DNA G+C content of strain RYG^T was calculated using a linear regression analysis of melting temperatures (T_m) against the DNA G+C contents of the reference strains. The G+C content was estimated to be 49.87±0.87 mol%.

The two members of the genus *Ohtaekwangia* were isolated from marine sands in Korea (Yoon *et al.*, 2011). Several of their chemotaxonomic features compared well with strain RYG^T, such as predominance of the fatty acids iso-$C_{15:0}$ and $C_{16:1}\omega5c$ and the major respiratory quinone MK-7. However, these chemotaxonomic features are very common amongst the class *Cytophagia* and have little value in distinguishing genera (Yoon *et al.*, 2011). Other similarities included cell size and morphology and pH and temperature for growth. The isolate and the reference strains were positive for the production of flexirubin-type pigments and catalase and oxidase, but negative for anaerobic growth. However, there were several major differences between strain RYG^T and the reference strains (Table 3), which included IPL content (strain RYG^T contained a major unique IPL), G+C content (50 versus 43–45 %), maximum NaCl for growth (1 versus 0.2 %) and gliding motility (positive versus negative). These differences, combined with the low 16S rRNA gene sequence similarity (93 %), between the isolate and its closest relatives lead us to propose a new genus to accommodate strain RYG^T, *Chryseolinea* gen. nov.

We previously isolated *Edaphobacter aggregans*, a member of the group I *Acidobacteria*, in the same manner as strain RYG^T, from a co-culture with a methanotroph (Koch *et al.*,

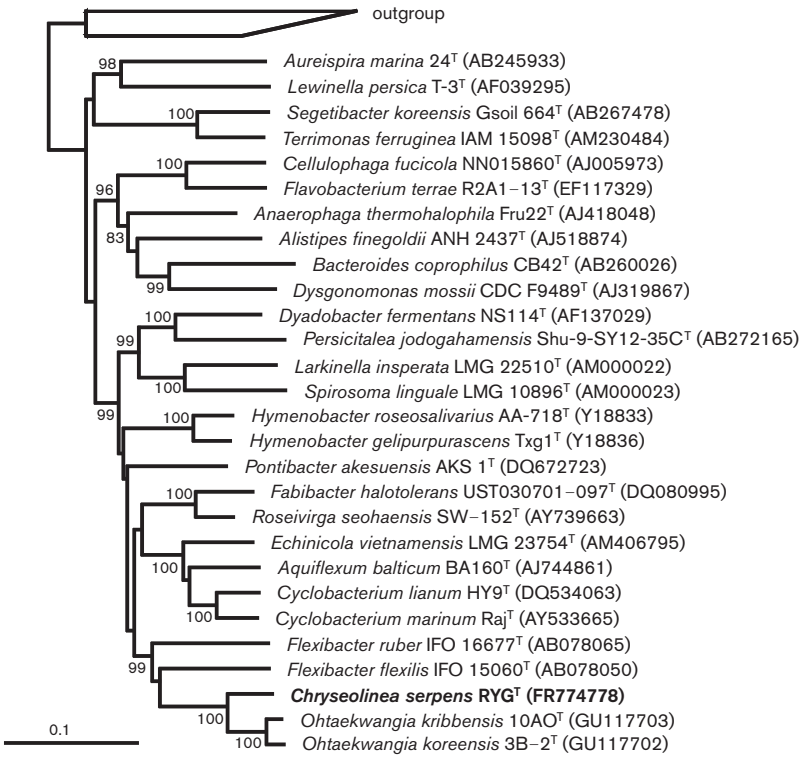

Fig. 2. Neighbour-joining phylogenetic tree (Jukes–Cantor correction) based on 16S rRNA gene sequences showing the position of strain RYG^T in relation to other representatives of the phylum *Bacteroidetes*. Bootstrap values (>80 %) based on 1000 resamplings are shown at branch nodes. *Methylohalobius crimeensis* 10Ki^T (GenBank accession number AJ581837), *Serratia marcescens* KRED (AB061685), *Acidobacterium capsulatum* 161^T (D26171), *Thermus aquaticus* YT-1^T (L09663) and *Verrucomicrobium spinosum* DSM 4136^T (X90515) were used as an outgroup. Bar, 0.1 changes per position.

2008). Neither this organism nor strain RYG^T is capable of growth on methanol, which is produced during methanotrophic growth and considered a major substrate for contaminants of methanotrophic cultures (Hanson & Hanson, 1996). However, the two strains are versatile heterotrophs and can grow on very nutrient-poor dilute medium. It is likely that these strains survive on trace contaminants in the mineral salts medium that is used to grow methanotrophs or, more likely, on traces of mono- and polysaccharides produced by the methanotrophs themselves, probably as exopolysaccharides. The methanotrophs with which these two heterotrophs were co-isolated, of the genera *Methylocella* and *Methylocapsa* in the family *Beijerinckiaceae*, have been shown to produce large amounts of exopolysaccharide (Dunfield *et al.*, 2003, 2010).

Description of *Chryseolinea* gen. nov.

Chryseolinea (Chry.se.o.li′ne.a. Gr. adj. *khruseos* golden; L. fem. n. *linea* a linen thread, a string, line; N.L. fem. n. *Chryseolinea* golden-coloured thread.

Gram-stain-negative, heterotrophic, obligately aerobic, gliding bacterium. The major cellular fatty acids are $C_{16:1}\omega5c$ and iso-$C_{15:0}$. The major respiratory quinone is menaquinone-7 (MK-7). The major polar lipids are phosphatidylethanolamine and two unknown polar lipids. Of the class *Cytophagia*, order *Cytophagales*, phylum *Bacteroidetes*. The type species is *Chryseolinea serpens*.

Description of *Chryseolinea serpens* sp. nov.

Chryseolinea serpens (ser′pens. L. n. *serpens* (nominative in apposition) a creeping thing, creeper, crawler, snake, serpent, referring to its shape and gliding motility).

In addition to the genus description, displays the following properties. Thin rods (0.4–0.6 μm in width and 2.0–5.5 μm in length). Multiplies by normal cell division. No resting stages are observed. Forms yellow swarming colonies. Flexirubin-type pigments are produced. Motile by gliding. Grows at pH 5.6–7.7, at 13–37 °C and with ⩽1 % NaCl. Grows on diverse mono- and disaccharides as well as some polysaccharides and few organic acids. Unable to grow on alcohols. Xylan, pectin and starch are degraded, but alginic acid and CM-cellulose are not. With GN2 MicroPlates, utilizes dextrin, α-D-glucose, L-proline, α-cyclodextrin, glycogen, *N*-acetyl-D-glucosamine, cellobiose, melibiose, methyl β-D-glucoside, raffinose, sucrose, trehalose, turanose, D-fructose, L-fructose, D-galactose, gentiobiose, α-lactose, lactulose, maltose, D-mannose, acetic acid, D-gluconic acid, DL-lactic acid, hydroxy-L-proline, L-threonine, glucuronamide, L-asparagine, L-aspartic acid, L-glutamic acid, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate; the remaining substrates are weakly utilized or not utilized. Anaerobic growth via fermentation or denitrification not observed.

The type strain is RYG^T (=DSM 24574^T=ATCC BAA-2075^T), which was isolated from brook sediment near

Table 3. Major characteristics distinguishing strain RYG^T from its closest phylogenetic neighbours

Strains: 1, *Chryseolinea serpens* gen. nov., sp. nov. RYG^T; 2, *Ohtaekwangia koreensis* 3B-2^T; 3, *Ohtaekwangia kribbensis* 10AO^T. Fatty acids analysis and IPL analysis were performed in this study; other data for strains 2 and 3 were taken from Yoon *et al.* (2011).

Characteristic	1	2	3
Cell dimensions (μm)			
Width	0.4–0.6	0.3–0.6	0.2–0.5
Length	2.0–5.5	1.0–5.0	1.5–7.5
Major fatty acids (%)*			
iso-$C_{15:0}$	17.4	37.1[a]	26.2[b]
$C_{16:1}\omega5c$	29.4	18.3[a]	19.5[b]
$C_{16:0}$	6.9	1.1[a]	1.8[b]
Putative sphingolipid IPL containing a PE head group*	+	−[a]	−[b]
Hydrolysis of:			
Starch	+	−	−
Enzyme activity (API ZYM)			
Valine arylamidase	+	−	+
α-Chymotrypsin	−	+	+
β-Glucosidase	−	+	+
α-Fucosidase	−	+	+
Gliding motility	+	−	−
Maximum NaCl for growth (%)	1	0.2	0.2
Temperature for growth (°C)			
Range	13–37	10–39	10–39
Optimum	25–30	30	30
pH for growth			
Range	5.6–7.7	5.5–9.0	5.5–8.5
Optimum	6.6–7.0	6.5–7.5	6.5–7.5
G+C content (mol%)	49.9	42.8	44.6

*Data from this study using *a*, *O. koreensis* KCTC 23018^T; *b*, *O. kribbensis* KCTC 23019^T.

Marburg, Germany. The DNA G+C content of the type strain is 49.9 mol% (T_m).

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