Glaciecola polaris sp. nov., a novel budding and prosthecate bacterium from the Arctic Ocean, and emended description of the genus *Glaciecola*

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Four strains of cold-adapted, strictly aerobic and facultative oligotrophic bacteria were isolated from polar seas and investigated using a polyphasic taxonomic approach. Two strains (LMG 21857^T and LMG 21854) derive from Arctic sea water whereas the other two strains (LMG 21855 and LMG 21858) were isolated from Antarctic sea water. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these strains belong to the γ-subclass of the *Proteobacteria* and are related to the genus *Glaciecola*, with 98·0–99·7 % sequence similarity to *Glaciecola mesophila* and 94·2–95·3 % sequence similarity to *Glaciecola punicea*, their nearest phylogenetic neighbours. Two strains (LMG 21855 and LMG 21858) were identified as *G. mesophila*, whereas DNA–DNA hybridization results and differences in phenotypic characteristics showed that the other two strains (LMG 21857^T and LMG 21854) constitute a novel species within the genus *Glaciecola*, with a DNA G+C content of 44·0 mol%. The isolates are Gram-negative, chemoheterotrophic, motile, rod-shaped cells that are psychrotolerant and moderately halophilic. Buds can be produced on mother cells and on prosthecae. Branch formation of prosthecae occurs. Whole-cell fatty acid profiles of the isolates are very similar and include $C_{16:0}$ and $C_{16:1}\omega7c$ as the major fatty acid components. On the basis of genotypic and phenotypic properties, a novel species of the genus *Glaciecola* is described, for which the name *Glaciecola polaris* sp. nov. is proposed, with isolate LMG 21857^T (=CIP 108324^T=ARK 150^T) as the type strain. An emended description of the genus *Glaciecola* is presented.

The genus *Glaciecola* was proposed by Bowman *et al.* (1998) for two groups of psychrophilic bacteria isolated from sea-ice diatom assemblages from the coastal areas of eastern Antarctica and forms a separate lineage within the γ-subclass of the *Proteobacteria*, being distantly related to *Alteromonas macleodii*. Recently, another species of the genus *Glaciecola* was described, *Glaciecola mesophila*, isolated from marine invertebrate specimens (Romanenko *et al.*, 2003). Many genera of this class of *Proteobacteria* (*Alteromonas*, *Pseudoalteromonas*, *Glaciecola*, *Idiomarina* and *Colwellia*) are common inhabitants of the marine part of the biosphere and have very diverse habitats, e.g. coastal and open-water areas, deep-sea and hydrothermal vents, marine sediments and sea ice (Mikhailov *et al.*, 2002).

In another study, we reported that seven Antarctic strains belong to a novel species within the genus *Alteromonas*, *Alteromonas stellipolaris* (Van Trappen *et al.*, 2004b). Together with the novel *Glaciecola* species described here, they all belong to the group of novel budding and prosthecate bacteria from the γ-subclass of the *Proteobacteria*. It is now evident that budding and prosthecate bacteria are abundant in marine and polar environments (Weiner *et al.*, 2000; Labrenz *et al.*, 1998, 1999). Moreover, bud and prostheca formation is a common strategy for rod-shaped bacteria to enhance their surface-to-volume ratio, thus

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 21857^T and LMG 21855 are AJ293820 and AJ548479, respectively.

Normalized rep-PCR profiles (GTG_5 primer) and a dendrogram derived from the UPGMA clustering of the profiles are available as supplementary material in IJSEM Online.

enabling efficient substrate uptake in oligotrophic habitats (van Gemerden & Kuenen, 1984).

During expeditions in the Arctic (Tan & Rüger, 1991) and Antarctic seas (Tan & Rüger, 1999), facultative oligotrophic and psychrophilic/psychrotolerant bacteria were isolated. These strains (173) have been previously analysed by means of the Biolog system (substrate utilization patterns) (Tan, 1997; Tan & Rüger, 1999), fatty acid analysis and 16S rRNA gene sequence analysis of representatives (Mergaert *et al.*, 2001). They belong to six metabolic groups and eight different fatty acid clusters containing 2–59 strains. In the meantime, additional strains (56) were isolated using the same methods and were also analysed using the Biolog system and fatty acid analysis. The novel strains belong to fatty acid clusters B, C, D, E and F (as delineated in Mergaert *et al.*, 2001), and three new fatty acid clusters (I, J and K; S. Van Trappen, unpublished results) were found. The genomic diversity of the 19 strains from fatty acid clusters E and F and two related unclustered strains was further investigated (see also Van Trappen *et al.*, 2004b) and these strains were arranged in similarity groups based upon the results of repetitive sequence-based (rep)-PCR fingerprinting using the GTG_5 primer (Versalovic *et al.*, 1991; Rademaker & de Bruijn, 1997; Rademaker *et al.*, 2000). Numerical analysis was carried out using the BIONUMERICS software package (Applied Maths; available at http://www.applied-maths.com), as described by the same authors. One Arctic cluster (LMG 21857^T, LMG 21854) and one Antarctic cluster (LMG 21855, LMG 21858), each containing two strains and belonging to FAA cluster F and having almost identical rep-PCR profiles, could be delineated (see supplementary figure in IJSEM Online); 16S rRNA gene sequence analysis revealed that they belong to the genus *Glaciecola* within the γ-subclass of the *Proteobacteria*.

Strains were isolated from sea water after enrichment in dialysis chambers as previously described (Tan, 1986, 1997). The strains investigated were LMG 21857^T (=CIP 108324^T =ARK 150^T) and LMG 21854 (=ARK 149), isolated from Arctic sea water, and LMG 21855 (=ANT 12a) and LMG 21858 (=ANT 12b), from Antarctic sea water. The reference strains *Glaciecola punicea* LMG 21426^T, *Glaciecola pallidula* LMG 21427^T and *G. mesophila* LMG 22017^T were included in some experiments. Strains were routinely cultivated on marine agar 2216 (Difco) at 20 °C for 48 h, or, for strains LMG 21426^T and LMG 21427^T, on marine agar 2216 at 10 °C for 6 days, or, for strain LMG 22017^T, on marine agar 2216 at 28 °C for 24 h, unless mentioned otherwise.

Small-scale DNA extracts were prepared using the method of Pitcher *et al.* (1989) and the almost-complete 16S rRNA gene sequences (1485 nt) of strains LMG 21857^T, LMG 21854, LMG 21855 and LMG 21858 were amplified by a PCR using conserved primers (Coenye *et al.*, 1999). PCR products were purified using a QIAquick PCR Purification kit (Qiagen) according to the instructions of the manufacturer. Sequence analysis was performed as described earlier (Van Trappen *et al.*, 2004a). Evolutionary distances were calculated using the algorithm of Jukes–Cantor (Jukes & Cantor, 1969) and a phylogenetic tree (shown in Fig. 1) was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the TREECON program (Van de Peer & De Wachter, 1994). Dendrograms obtained using maximum-parsimony and maximum-likelihood analyses showed essentially the same topography (data not shown).

The 16S rRNA gene sequences of the two Arctic strains (LMG 21857^T and LMG 21854) are identical (100 % sequence similarity) and show 98·0 % similarity to *G. mesophila*, 94·2 % to *G. punicea* and 93·5 % to *G. pallidula*, whereas the sequences of the Antarctic strains (LMG 21855 and LMG 21858), which are also identical to each other, show 99·7 % sequence similarity to *G. mesophila*, 95·3 % to *G. punicea* and 94·9 % to *G. pallidula*. The sequence similarity between the Arctic and Antarctic strains is 98·4 %. The phylogenetic tree in Fig. 1 illustrates the phylogenetic relationships of the polar isolates within the genus *Glaciecola*. Strains LMG 21855 and LMG 21858 are very closely related to *G. mesophila*, whereas strains LMG 21857^T and LMG 21854 form a distinct branch supported by a high bootstrap value.

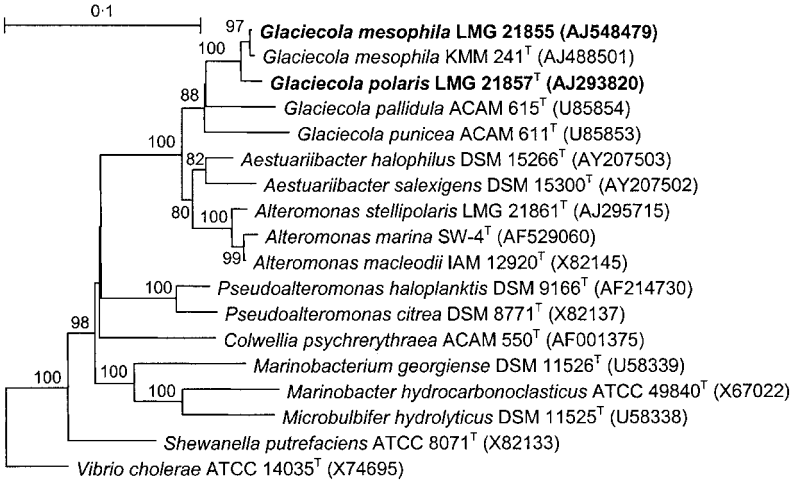

Fig. 1. Neighbour-joining dendrogram showing the estimated phylogenetic relationships of the Arctic and Antarctic isolates and other marine chemoheterotrophs of the γ-subclass of the *Proteobacteria*. Bootstrap values (percentages of 500 replicates) greater than 70 % are shown. The GenBank/EMBL/DDBJ accession number for each reference strain is shown in parentheses. Bar, 1 nucleotide substitution per 10 nucleotides.

The genomic relatedness between strains LMG 21857^T, LMG 21855 and the most closely related strains, *G. mesophila* LMG 22017^T and *G. punicea* LMG 21426^T, was determined by DNA–DNA hybridizations. DNA was prepared according to the method of Pitcher *et al.* (1989); DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. The hybridization temperature was 35 °C and reciprocal experiments were performed for every pair of strains. The hybridization level between strain LMG 21857^T and *G. mesophila* LMG 22017^T and *G. punicea* LMG 21426^T was 17·2 and 4·0 %, respectively, whereas the DNA–DNA binding value between LMG 21857^T and LMG 21855 was 23·4 %. The hybridization level between strain LMG 21855 and *G. mesophila* LMG 22017^T and *G. punicea* LMG 21426^T was 67·7 and 6·0 %, respectively. Differences between reciprocal experiments were less than 10 %. DNA–DNA hybridizations between strains of the same rep-PCR cluster were not performed since Versalovic *et al.* (1994) have shown that strains with the same rep-PCR profile are always closely related (as confirmed by several authors, e.g. Rademaker & De Bruijn, 1997). These results suggest that the two Arctic isolates are genotypically distinct from *G. mesophila* and *G. punicea* (their closest neighbours in phylogenetic terms) and constitute a novel species within the genus *Glaciecola*. The two Antarctic isolates are closely related to *G. mesophila*, showing a DNA–DNA reassociation value near 70 %, which is generally accepted as the threshold for species delineation (Wayne *et al.*, 1987).

DNA G+C contents of the Arctic and Antarctic strains were determined using an HPLC method, as described by Van Trappen *et al.* (2003). The DNA G+C contents of strains LMG 21857^T, LMG 21854, LMG 21855 and LMG 21858 are 44·2, 43·6, 43·9 and 44·2 mol%, respectively. These values are consistent with the G+C content of the genus *Glaciecola*, which ranges between 40 and 46 mol% (Bowman *et al.*, 1998).

The cellular fatty acid patterns of the polar strains are based on the data generated by Mergaert *et al.* (2001) or were determined as described by the same authors. The Arctic strains show very similar fatty acid profiles and the mean composition is as follows: 3·1 % $C_{12:0}$, 5·5 % $C_{12:0}$ 3-OH, 4·1 % $C_{14:0}$, 2·0 % $C_{15:0}$, 23·3 % $C_{16:0}$, 1·7 % $C_{16:0}$ 2-OH, 2·0 % $C_{16:1}$ 2-OH, 2·6 % $C_{17:1}\omega8c$, 4·8 % $C_{18:1}\omega7c$, 1·4 % $C_{18:0}$ 10-methyl and 41·7 % summed feature 3, which comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$. The Antarctic strains have fatty acid patterns very similar to those of strains KMM 241^T and KMM 642 (*G. mesophila*), with $C_{16:0}$, $C_{16:1}\omega7c$, $C_{17:1}\omega8c$ and $C_{18:1}\omega7c$ as the dominant fatty acids. Hydroxylated fatty acids and iso-branched fatty

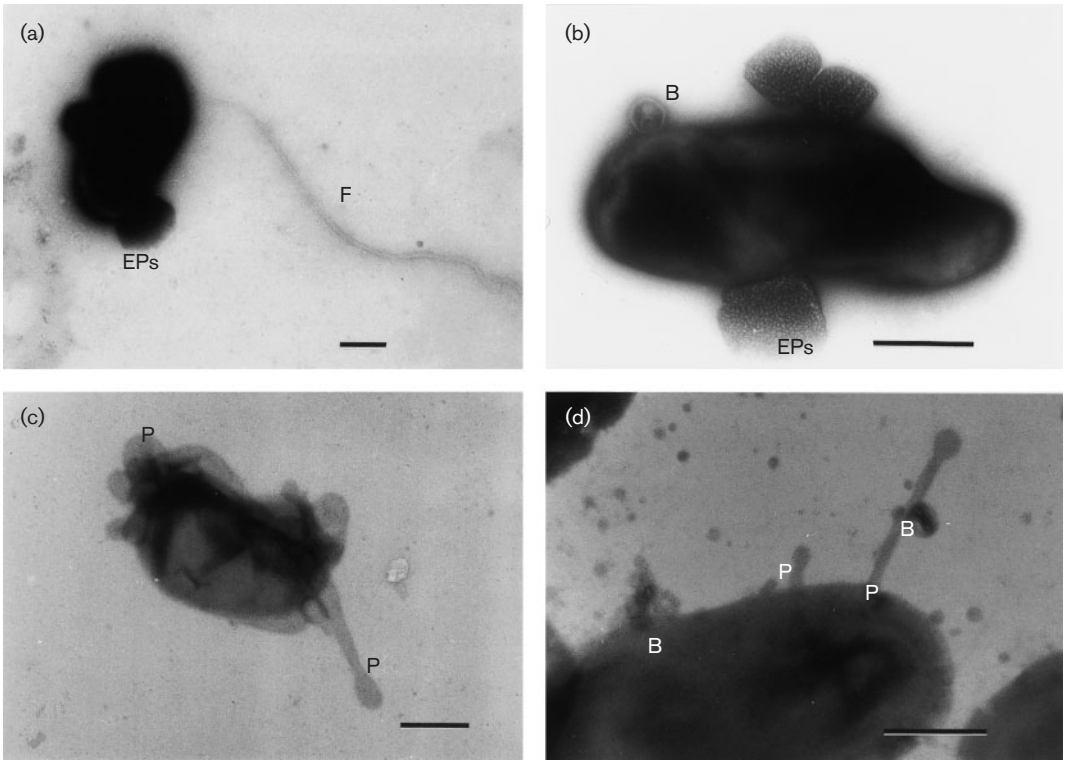

Fig. 2. Electron micrographs of negatively stained preparations of cells of strains LMG 21855 (a–c) and LMG 21858 (d), showing flagella (F), prosthecae (P), buds (B) and extracellular products (EPs). Colonies used for analysis were grown on PYG agar at 12 °C for 7 days. Cells were stained with 1 % (w/v) uranyl acetate in 0·4 % (w/v) sucrose. Bars, 300 nm.

acids were also present as minor components or at trace levels in the Arctic strains. The fatty acid profiles of the polar strains clearly resemble those determined for other marine genera of the γ-subclass of the *Proteobacteria*, e.g. *Alteromonas*, *Pseudoalteromonas* and *Glaciecola* (Ivanova *et al.*, 2000).

The growth of the strains at different temperatures (5–37 °C) was tested on marine agar 2216, whereas salt tolerance was tested on R2A agar (Difco), supplemented with 1–20 % (w/v) NaCl, at 20 °C. Biochemical characteristics were determined using standard protocols (Smibert & Krieg, 1994; West & Colwell, 1984; Reichenbach & Dworkin, 1981; Bowman *et al.*, 1998; Van Trappen *et al.*, 2003) and API kits (API 20E, API 20NE, API ZYM and API 32ID; bioMérieux). Bacterial suspensions were made in sterile, chilled sea water, and marine agar was used as the basal medium. For Biolog GN2 microplates, the bacteria were grown on peptone/yeast extract/glucose (PYG) agar at 20 °C for 5 days; the cells were harvested and suspended in 'inoculating fluid'. The salinity of the 'inoculating fluid' was adjusted to 26 parts per thousand with NaCl. The microplates were incubated at 20 °C and substrate utilizations were measured after 3–28 days at 590 nm with an eight-channel photometer (Spectra 2; SLT Labinstruments).

The Antarctic strains are Gram-negative, rod-shaped cells (0·4 μm in width and 2–3 μm in length) and are flagellated. Buds and prosthecae can be produced (Fig. 2). The strains are able to grow between 5 and 30 °C, whereas no growth occurs at 37 °C; growth is supported on R2A agar with up to 10 % (w/v) NaCl. The colonies have a mucoid consistency, show reactions very similar to those of strains KMM 241^T and KMM 642 of *G. mesophila* and reduce nitrate. They differ from these strains in the degradation of agar and the utilization of D-fructose, D-trehalose, L-glutamate and L-proline, and the Antarctic strains grow at 4 °C and in 10 % (w/v) NaCl (see Table 1). Antarctic strains LMG 21855 and LMG 21858 are identified as *G. mesophila* since there are only a few phenotypic differences, which could be due to the different protocols used, and DNA–DNA hybridization results together with the 16S rRNA gene sequence similarities also support the notion that these Antarctic isolates are very closely related to *G. mesophila*.

The cells of the Arctic strains are Gram-negative, rod-shaped (0·4 μm in width and 2–3 μm in length) and polarly or subpolarly flagellated. Buds can be produced on mother cells or on prosthecae (Fig. 3). Prostheca formation is peritrichous; prosthecae can be branched. The strains are able to grow between 5 and 30 °C, whereas no growth occurs at 37 °C. Growth is supported on R2A agar with up to 10 % (w/v) NaCl, indicating that they are moderately halophilic and psychrotolerant. This is in contrast to *G. punicea* and *G. pallidula*, which have an absolute requirement for sea water and are psychrophilic (Bowman *et al.*, 1998), and *G. mesophila*, which is slightly halophilic and mesophilic (Romanenko *et al.*, 2003). The strains are aerobic, chemoheterotrophic bacteria and there is no evidence for their growth under anaerobic conditions. Only strain LMG 21854 produces colonies with a mucoid consistency. The Arctic strains are positive for precipitation on egg-yolk agar and show the typical properties of the genus *Glaciecola* (see the species description).

Table 1. Phenotypic characteristics that differentiate *G. polaris* sp. nov. from its nearest phylogenetic neighbours

Species: 1, *G. polaris* sp. nov.; 2, *G. mesophila*; 3, *G. punicea*; 4, *G. pallidula*. Data are from Bowman *et al.* (1998), Romanenko *et al.* (2003) and this study. Symbols: +, positive; −, negative; V+, variable between strains, type strain positive; V−, variable between strains, type strain negative. All strains were positive for the following tests: motility, sodium ion requirement for growth, oxidase, catalase, growth at 7–20 °C and growth in 1–6 % (w/v) NaCl. All strains were negative for growth at 37–40 °C, the indole reaction, arginine dihydrolase, chitin hydrolase and for utilization of L-arabinose, citrate, L-histidine, L-ornithine, L-threonine and *N*-acetylglucosamine.

Characteristic	1	2	3	4
Pigmentation	−	−	Pink–red	Pale pink
Growth at 25 °C	+	+	+	−
Growth in 10 % (w/v) NaCl	+	V−	−	−
Hydrolysis of:				
Egg yolk	+	−	−	−
Starch	+	+	−	V+
Aesculin	+	+	V+	−
DNA	+	+	−	−
β-Galactosidase	+	+	+	−
Nitrate reduction	−	+	−	−
Utilization of:				
D-Glucose, D-mannitol, cellobiose	+	+	−	−
Sucrose, maltose	+	V+	−	−
D-Galactose	+	V+	−	−
D-Fructose, D-trehalose	+	V+	−	−
D-Mannose	+	V+	−	−
Glycerol	−	−	−	+
Acetate	+	−	−	+
Glycogen, dextrin	+	+	−	+
DL-Lactate	−	−	−	+
Propionate	+	−	−	−
L-Glutamate	+	V+	−	+
L-Malate	−	−	+	−
DNA G+C content (mol%)	44	44	44–46	40

On the basis of this polyphasic taxonomic analysis, the Arctic strains can be clearly differentiated from the other species within the genus *Glaciecola* (see Table 1) and can be assigned to a novel species, for which the name *Glaciecola polaris* sp. nov. is proposed.

Emended description of the genus *Glaciecola* Bowman *et al.* 1998 emend. Van Trappen *et al.*

The description is as described by Bowman *et al.* (1998), with the following additional morphological features. When

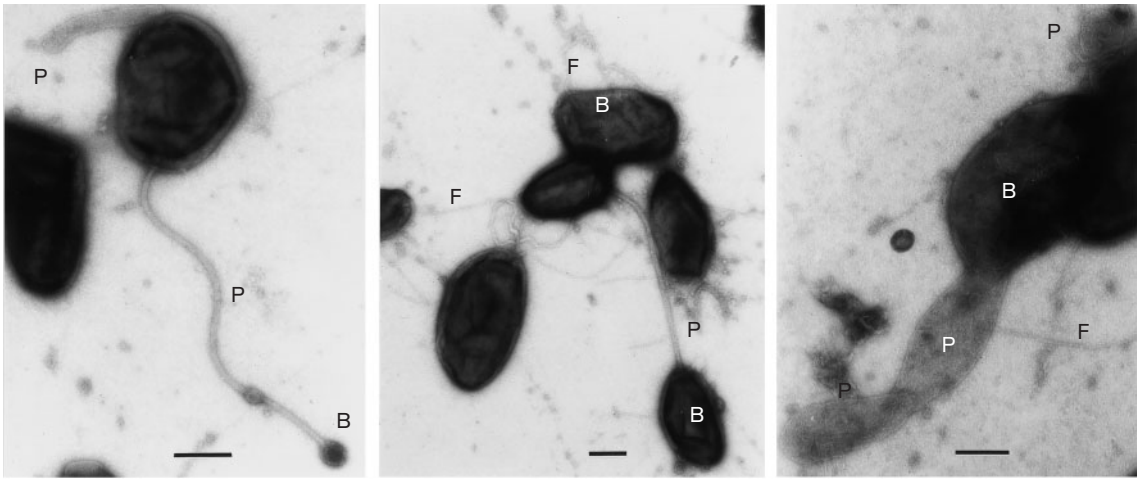

Fig. 3. Electron micrographs of negatively stained preparations of cells of strain LMG 21857^T, showing flagella (F), prosthecae (P) and buds (B). For methods, see the legend to Fig. 2.

grown on marine or PYG agar at low temperatures (12–20 °C) for 3 days or more, some strains can produce buds and prosthecae (see Figs 2, 3 and 4).

Description of *Glaciecola polaris* sp. nov.

Glaciecola polaris (po.la′ris. N.L. fem. adj. *polaris* polar, referring to the origin of the strains in the Arctic Ocean).

Cells are Gram-negative, short rods (0·4 × 2–3 μm) that are motile by means of a polar or subpolar flagellum. Buds can be produced on mother cells or on prosthecae. Prostheca formation is peritrichous and prosthecae can be branched (Figs 2, 3 and 4). They form non-pigmented, circular, low convex, shiny and opaque colonies that are not adherent to agar and which have entire margins and a diameter of 1–4 mm on marine agar 2216 plates after 7 days incubation at 20 °C. Growth occurs on marine and PYG agar and there is a slight growth on nutrient agar, but there is no growth on trypticase soy agar or R2A agar. The temperature range for growth is 5–30 °C, whereas no growth occurs at temperatures of 37 °C or above; growth is observed on R2A agar with up to 10 % (w/v) NaCl, indicating that the cells are moderately halophilic and psychrotolerant. There is no evidence for growth under anaerobic conditions, and the catalase and cytochrome oxidase tests are positive. Tests positive for the degradation of starch, aesculin and DNA, and precipitation on egg-yolk agar occurs. β-Galactosidase activity is detected for both strains. They are able to utilize α-cyclodextrin, dextrin, glycogen, Tween 40, Tween 80, D-arabitol, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-raffinose, sucrose, D-trehalose, D-sucrose, furanose, methylpyruvate, acetic acid, β-hydroxybutyric acid, propionic acid, L-alanine, L-alanyl-glycine, L-glutamic acid, glycyl-L-glutamic acid, L-leucine, L-pyroglutamic acid and salicin. Both strains are negative in tests for indole and acetoin production, in the Voges–Proskauer test and in tests for citrate utilization, hydrolysis of urate, nitrate reduction and the production of hydrogen sulfide. No growth is observed on arabinose, *N*-acetylglucosamine, caprate, adipate, malate, citrate, phenylacetate, L-fucose, D-sorbitol, valerate, histidine, 2-ketogluconate, 3-hydroxybutyrate, 4-hydroxybenzoate, rhamnose, D-ribose, inositol, itaconate, suberate, malonate, DL-lactate, 5-ketogluconate, 3-hydroxybenzoate, L-serine, alaninamide, L-threonine or glycerol. No acids are produced from the carbohydrates glucose, mannitol, inositol, sorbitol,

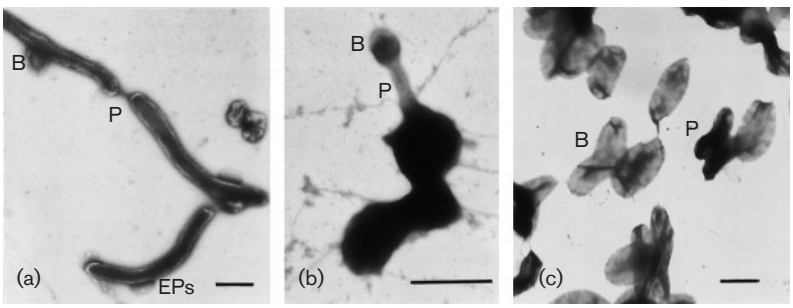

Fig. 4. Electron micrographs of negatively stained preparations of cells of *G. punicea* LMG 21426^T (a), *G. pallidula* LMG 21427^T (b) and *G. mesophila* LMG 22017^T (c), showing prosthecae (P), buds (B) and extracellular products (EPs). Colonies used for analysis were grown on PYG agar at 20 °C for 3 days (*G. mesophila*) or on marine agar at 12 °C for 21 days (*G. punicea*) or 12 days (*G. pallidula*), respectively. Cells were stained with 1 % (w/v) uranyl acetate in 0·4 % (w/v) sucrose. Bars, 1000 nm.

rhamnose, sucrose, melibiose, amygdalin or arabinose and tests for the degradation of alginate, chitin, casein, gelatin and urea are negative. There is no arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase or tryptophan deaminase activity. There is no activity for the enzymes lipase (C14), cystine arylamidase, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. For both strains, low activity (score 1) is obtained for valine arylamidase, trypsin, α-glucosidase and β-glucosidase, medium activity (score 2 or 3) is observed for esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-galactosidase, and high activity (score 4 or 5) is observed for alkaline phosphatase and leucine arylamidase. Cells contain fatty acids $C_{16:0}$ and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) as the main constituents. The DNA G+C content is 44·0 mol%.

The type strain is LMG 21857^T (=ARK 150^T=CIP 108324^T). Isolated from the Arctic Ocean.

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