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ORIGINAL PAPER

Novel ultramicrobacterial isolates from a deep Greenland ice core represent a proposed new species, *Chryseobacterium greenlandense* sp. nov.

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Abstract Three novel orange, ultramicrobacterial isolates, UMB10, UMB14, and UMB34^T were isolated from enrichment cultures inoculated with a melted 3,043 m deep Greenland ice core sample. Phylogenetic analysis of the 16S rRNA gene sequences indicated that the isolates belonged to a single species within the genus *Chryseobacterium*. They were most closely related to *Chryseobacterium aquaticum* (99.3%), *Chryseobacterium soli* (97.1%), and *Chryseobacterium soldanellicola* (96.9%). Genomic hybridization showed low levels of relatedness between UMB34^T and *C. aquaticum* and *C. soldanellicola* (19–30%) and *C. soli* and *Chryseobacterium jejuense* (45–56%). Comparative genomic fingerprinting analysis using the enterobacterial repetitive intergenic consensus (ERIC) sequence showed nearly identical banding patterns for the three isolates and these patterns were distinct from those of *C. aquaticum*, *C. soldanellicola, C. soli*, and *C. jejuense.* The cells were short rods, lacked flagella, had cell volumes of <0.1 μm^3, formed buds and smaller protrusions (blebs), produced copious extracellular material and a flexirubin type pigment. UMB34^T produced acids from carbohydrates and utilized glucose and maltose although it did not assimilate mannose. The DNA G + C was 39.6–41.6 mol%. Based on the differences from validly named *Chryseobacterium* species, it was concluded that these isolates represent a new species for which the name, *Chryseobacterium greenlandense* is proposed. The type strain is UMB34^T (=CIP 110007T = NRRL B-59357).

Keywords *Chryseobacterium greenlandense* · *Flavobacteriaceae* · Ultramicrobacteria · Glacial isolates · Ice core

Introduction

Frozen environments, including glaciers, ice sheets, permafrost, sea ice, and snow constitute a significant portion of the earth's biosphere. Although these habitats may seem inhospitable, some microorganisms do survive and perhaps thrive under these extreme conditions (Cowan and Tow 2004; Priscu and Christner 2004; Priscu et al. 2007; Miteva 2008). Our research has focused on the microbiology of the Greenland ice sheet, the second largest on Earth, and an exceptional habitat in which the climatic and microbiological records are chronologically preserved for hundreds of thousands of years. Initial studies of melted ice from a 3,043 m deep Greenland Ice Sheet Project 2 (GISP2) core sample (Sheridan et al. 2003b; Miteva et al. 2004) showed the presence of a phylogenetically diverse population and intriguingly, many of the cells were ultrasmall (<1 μm). Ultramicrobacteria, defined as cells that maintain a volume of less than 0.1 μm^3 despite environmental fluctuations, are plentiful in aquatic and terrestrial environments (Cavicchioli and Ostrowski 2003). Although these ultrasmall-celled organisms may predominate in many habitats, they are extremely difficult to cultivate and few species have been validly described.

In order to increase our understanding of these ultra small-celled organisms, we isolated some ultramicrobacteria from

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successive enrichments of 0.2 μm filtrates of melted ice from the same GISP2 sample and long-term incubations at low temperatures. Geological dating of the ice core indicates that these cells had been trapped in the ice for at least 120,000 years and possibly much longer if the cells originated from the underlying permafrost. After inoculating R2A plates from a low salt mineral broth with formate and an R2B culture, we obtained three orange-pigmented Gram-negative ultramicrobacterial isolates that we designated UMB10, UMB14 and UMB34^T (Miteva and Brenchley 2005). Analysis of 16S rRNA gene sequences indicated that the isolates were related to species in the genus *Chryseobacterium*, a member of the family *Flavobacteriaceae*, which contains about 80 highly diverse genera. The genera *Chryseobacterium* and *Bergeyella* were created and the genus *Empedobacte*r "revived" to update an "ill-defined" genus, *Flavobacterium* (Vandamme et al. 1994). Currently, there are 41 validly named *Chryseobacterium* species isolated from widely divergent habitats including fresh, salt and wastewater, marine sediments, fish, dairy products, soil, plant material and a beer production plant (http://www.bacterio.cict.fr/c/chryseobacterium.html). In addition, many clinical specimens have been obtained from fish, frogs, and humans. Some *Chryseobacterium* strains may cause disease in fish or frogs and some are potential opportunistic pathogens for humans (Bernardet et al. 2005, 2006).

In this paper, we present the results of our polyphasic characterization of the three novel ice core isolates. Based on the morphological, phylogenetic and physiological characteristics, these ultramicrobacterial isolates represent a new species, for which we propose the name, *Chryseobacterium greenlandense* sp. nov.

Materials and methods

Sampling and isolation

The 3,043 m deep core sample from the GISP2 was aseptically sampled as described previously (Sheridan et al. 2003b). The melted Greenland glacial ice was subsequently used in a filtration–cultivation scheme that enriched for ultrasmall cells (Miteva and Brenchley 2005). Briefly, the three isolates were obtained as follows: melted ice was passed through 0.2 μm filters and the filtrates used to inoculate anaerobic, salt medium, MM1 with formate (Miteva et al. 2004), and incubated for 7 months at 5°C. Samples from the first enrichment were filtered and filtrates were used to inoculate either the same medium or R2B and incubated for an additional 18 days at 5°C. Aliquots from the enrichments were plated on low nutrient agar medium, R2A, and incubated for another 2–4 months at 5°C. Concurrently, enrichments inoculated with unfiltered melted ice also were incubated for the same amounts of time and analyzed in parallel. UMB14 was isolated from a plate inoculated with a filtered ice/culture sample and UMB10 and UMB34^T from unfiltered ice/cultures.

Physiological, biochemical, and chemotaxonomic characterization

Physiological and biochemical characterizations were performed using the isolate UMB34^T. Growth temperature range was examined aerobically on R2A and Tryptic Soy Agar without added dextrose (TSA) from 1 to 42°C. Specific growth rate in Tryptic Soy Broth without added dextrose (TSB) was assessed for cultures shaken at 120 rpm at 4, 12, 18, 25, 30, 35, 37, and 40°C by monitoring the change in turbidity with a Summerson–Klett Meter (New York, USA). In addition, growth under anaerobic conditions was determined by incubating cultures on TSA in a GasPak pouch (Becton–Dickinson and Company, ML, USA) at 25°C and under microaerophilic conditions generated by CampyPak envelopes (Becton–Dickinson and Company) at 30°C. Swarming activity was detected on 0.18× R2A. Five microlitre suspensions of UMB34^T were placed in the center of the plates incubated at 25°C in a humid atmosphere for 6 days and the growth patterns were observed.

Carbohydrate utilization was determined using API 20 NE strips (bioMérieux, North Carolina, USA), incubated at 25°C and the results recorded after 1, 2, and 3 days. Acid production from carbohydrates was assessed using API 50 CH strips. Colonies were suspended in CHB/E medium (bioMérieux, NC, USA) and the inoculated test medium was placed in the tubes with no oil overlay. The strips were incubated at 25°C and examined after 1, 2, 3, 6, 7, 10, and 14 days. The production of acids from glucose and maltose was confirmed by growing the cells in a modified Hugh and Leifson oxidation/fermentation medium at 25°C. In addition to the API 20 NE tests for hydrolysis of gelatin, esculin, PNPG, and urea, degradation of other biopolymers was checked by traditional methods. UMB34^T was inoculated onto different plates with substrates including Tween 80, lecithin (egg yolk agar) (Smibert and Krieg 1981), casein (Sokol et al. 1979) and starch (de Prada et al. 1996). The isolate also was tested for cleavage of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in Luria–Bertani agar and growth on MacConkey's, marine, and cetrimide agars. Growth on R2A containing 2, 5, 7, 10, and 20% NaCl and TSA containing 5, 7, and 10% NaCl also was tested.

Antibiotic sensitivity of UMB34^T was determined by disc diffusion (BD BBL sensi-discs) after 72 h at 30°C on TSA.

Discs containing the following concentrations of antibiotics were used: ampicillin (10 μg), bacitracin (10 IU), ciprofloxacin (5 μg), erythromycin (15 μg), nalidixic acid (30 μg), neomycin (30 μg), penicillin (10 IU), rifampicin (5 μg), streptomycin (10 μg), and vancomycin (30 μg).

To determine if the strain had a flexirubin type pigment, observation of the color change caused by the addition of KOH and the subsequent re-acidification was done as described by Bernardet et al. (2002).

Fatty acid determinations were done on cells of UMB34^T, *C. aquaticum* KCTC 12483^T, *C. soldanellicola* KCTC 12382^T, and *C. soli* DSM 19298^T grown on TSA plates for 48 h at 28°C. Colonies scraped from the plates were washed with sterile ultrapure water and the pelleted cells frozen at −20°C and sent to Microbial ID (Delaware, USA) for fatty acid methyl ester analysis.

Electron microscopy

Cells from the isolates were cultivated on R2A at 25°C for 72 h and the growth scraped from the agar was re-suspended in water and used for Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The samples for SEM were processed and images taken as described previously (Miteva and Brenchley 2005). TEM sectioned samples were processed as described earlier (Sheridan et al. 2003a). To prepare negatively stained samples, the cells were fixed with 2% glutaraldehyde, applied to carbon/formvar supported grids, and then stained with aqueous 2% uranyl acetate. Cell size was determined from SEM images and cell volume calculated by the method of Janssen et al. (1997).

Genetic analyses

Genomic DNA from UMB10, UMB14, UMB34^T, *C. aquaticum* KCTC 12483^T, *C. soldanellicola* KCTC 12382^T, *C. soli* DSM 19298^T and *C. jejuense* DSM 19299^T cells was extracted using the Pure Gene kit (Gentra systems, Inc., MN, USA). The G + C content (mol%) for purified DNA from cells of UMB34^T was determined by the method of (Gonzalez and Saiz-Jimenez 2002). 16S rRNA genes for UMB34^T, UMB14, and UMB10 were amplified with 8F-1492R primers using PuRe Taq Ready-to-go PCR beads (GE Healthcare, NJ, USA). The purified products were sequenced in both directions using additional 704F and 907R primers on an ABI 7300 sequencer (Miteva et al. 2004). The phylogenetic relationship of the isolates to other closely related species in the genus was performed using the PAUP program (Swofford 2002) after employing Clustal X (Thompson et al. 1997) for multiple sequence alignments. The relatedness of UMB34^T to *C. aquaticum* KCTC 12483^T, *C. soldanellicola* KCTC 12382^T, *C. soli* DSM 19298^T and *C. jejuense* DSM 19299^T was assessed by DNA–DNA hybridization. Hybridizations of sonicated genomic DNA samples were performed at 55°C in 2× SSC buffer, the hybrids detected fluorometrically, and the percent binding calculated by the method of De Ley et al. (1970). Comparative genomic fingerprinting used a single primer, the enterobacterial repetitive intergenic consensus (ERIC) sequence, according to the protocol described previously (Miteva and Brenchley 2005).

Results and discussion

Morphological, physiological, and chemotaxonomic characteristics

UMB10, UMB14 and UMB34^T formed round, convex, colonies within 2–3 days at 25°C that were yellow (R2A) to dark orange (TSA) 1–2 mm in diameter. The isolates also grew on 0.1× R2A although more slowly. The pigment was of the flexirubin type as evidenced from the brown coloration observed after the addition of 20% KOH and the subsequent return to yellow/orange after re-acidification with 1% sulfuric acid. These pigments are characteristic of *Chryseobacterium* species (Bernardet et al. 2002, 2006; Bernardet and Nakagawa 2006) and are produced by most species in the genus. Although the colonies typically had entire edges, after incubation for several weeks, especially on low-nutrient medium such as R2A, the colony edges appeared rhizoid.

The cells of UMB34^T and the other two isolates were Gram-negative, non-flagellated short rods, 0.6–1.2 μm × 0.4–0.5 μm, with an average volume of 0.08 μm^3, which is below the defined limit (0.1 μm^3) for ultramicrobacteria (Cavicchioli and Ostrowski 2003) (Fig. 1). The cell width is similar to those reported for the closely related validly named *Chryseobacterium* species, *Chryseobacterium indoltheticum, Chryseobacterium balustinum, Chryseobacterium scophthalmum, Chryseobacterium piscium* and *C. aquaticum* isolated from marine mud, fish, or water which usually averaged 0.4–0.5 μm or more; however, those cells tended to be longer, 0.8–1.0 to 1.5–2.0 μm (Campbell and Williams 1951; Holmes et al. 1984; Mudarris et al. 1994; de Beer et al. 2006; Kim et al. 2008). Relatives isolated from other environments, *C. soli*, from soil (Weon et al. 2008) and *Chryseobacterium gambrini*, and *Chryseobacterium ureilyticum*, from a beer bottling plant (Herzog et al. 2008), had wider and longer cells than did the isolates.

One intriguing morphological characteristic was the presence of buds (white arrows) on cells as seen in the TEM micrograph of UMB34^T (Fig. 1c) and SEM micrographs of UMB10 (Fig. 1b). To our knowledge, these

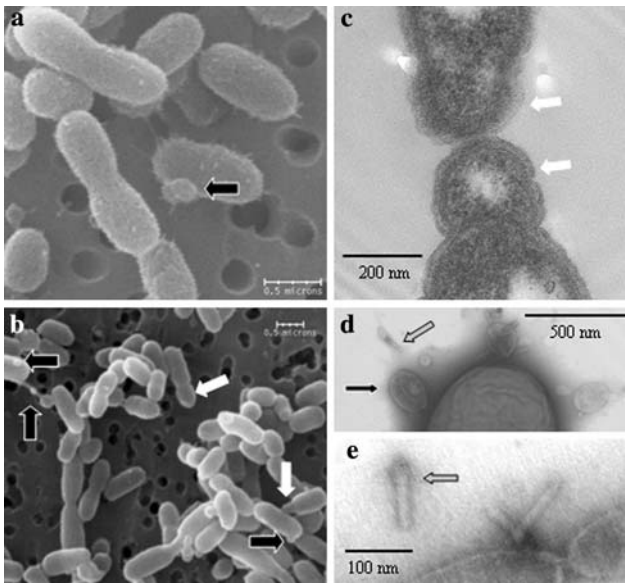

Fig. 1 Electron microscopy of cells grown on R2A at 25°C. **a** Scanning electron micrograph of UMB34^T cells. *Black arrow* indicates a protrusion or "bleb" on a cell. **b** Scanning electron micrograph of UMB10 cells. *Black arrows* indicate blebs and *white arrows* point to bud-like structures. **c–e** Transmission electron micrographs of UMB34^T cells. In **c,** the *white arrows* indicate buds. In **d** and **e,** there are small "rods" indicated by the *gray arrows* that may be phage particles. Also, in **d,** a *black arrow* points to a bleb adjacent to the cell

structures have not been observed with other *Chryseobacterium* species. One distant relative in the *Flavobacteriaceae* family, *Formosa agariphila* KMM 3901^T, an aerobic, gliding, halotolerant rod-shaped bacterium isolated from the Sea of Japan, formed buds (Nedashkovskaya et al. 2006).

A second unique characteristic was the occurrence of small protrusions or blebs on the surface of cells and on the interconnecting "threads" between cells as indicated by the black arrows in the SEM micrographs of UMB34^T (Fig. 1a) and UMB10 (Fig. 1b) and TEM micrograph of UMB34^T (Fig. 1d). It is unclear if the blebs and the buds are the same structures. The blebs may be immature buds, or these formations may have different functions. Examination of the TEM micrographs (Fig. 1d, e) of UMB34^T shows thin rod structures (gray arrows) that possibly are phage particles and the blebs may be implicated in their release. A third possibility may be that the blebs have a function in the production of extracellular material and/or cell aggregation. Many Gram-negative bacteria form outer membrane vesicles (MVs), 50–250 nm in diameter by "bulging and pinching off" the membrane. The MVs appear morphologically to resemble the blebs observed on UMB34^T. The functions of the MVs are being studied but one role may be as a "major component" of biofilms (Mashburn-Warren et al. 2008).

Appraisal of the SEM micrographs of UMB34^T and UMB10 showed considerable extracellular material on the cell surfaces and between cells (not presented). UMB34^T produced exopolymeric substances visible in both liquid and solid media and cells of UMB34^T adhered to glass surfaces especially when cultured in semisolid media in tubes. Other *Chryseobacterium* species produce exopolymeric substances. An example is *C. aquaticum*, the nearest validly named relative of the isolates, that formed mucoid colonies after 2 weeks (Kim et al. 2008). The gliding bacterium, *Formosa agariphila* KMM 3901^T produced "fibrillar threads" and slime that are suggested to be involved in forming biofilms and "aggregates in liquid cultures" (Nedashkovskaya et al. 2006).

Bernardet and Nakagawa (2006) noted that gliding motility might also be related to adherence of Flavobacteria to surfaces, the production of exopolymers and the formation of biofilms. Gliding motility is a characteristic that has been used to distinguish some genera in the family *Flavobacteriaceae* from others such as *Chryseobacterium* a genus that has no motile species (Bernardet et al. 2002, 2006; Bernardet and Nakagawa 2006). Formation of colonies with rhizoid edges may indicate cells are able to translocate in this fashion (Bernardet and Nakagawa 2006; Bernardet et al. 2002). When suspensions of UMB34^T were inoculated onto plates with <0.3% agar, the growth spread 10–12 mm in 72 h at 25°C suggesting that under certain conditions the cells exhibited swarming-like behavior. Kaiser (2007) suggested that swarming be defined as spreading over a "suitably moist solid medium" and this movement may be accomplished in different ways with or without flagella. For example, *Flavobacterium johnsoniae* has filaments on the cell surface that have been suggested as "mediating" the gliding by these cells (Liu et al. 2007).

UMB10, UMB14 and UMB34^T isolates grew aerobically on R2A between 1–2 and 37°C although UMB34^T did not form isolated colonies on TSA at 1–2°C after 5 weeks. In addition, there was no growth of UMB34^T on TSA at 42°C. The temperature range of growth for the isolates was greater than for some of their closest relatives (Table 1). The shortest generation time of isolate UMB34^T was 2 h at 30°C in TSB and there was no growth at 40°C. These growth parameters differed from those of more distant relatives isolated from cold habitats. *Sejongia antarctica*, *Sejongia jeonii*, and *Sejongia marina* (Yi et al. 2005; Lee et al. 2007), reassigned to the *Chryseobacterium* genus (Kämpfer et al. 2009a), isolated from Antarctic soil, moss and seawater, did not grow above 25–31°C and had temperature "optima" of about 15–21.5°C. Although the fastest growth rate for UMB34^T occurs at a more elevated temperature than its Antarctic relatives, some psychrophiles, and mesophiles to a lesser extent, produce greater biomass, and have greater enzymatic activity at

Table 1 Characteristics that distinguish UMB34^T from *Chryseobacterium aquaticum* KCTC 12483^T, *Chryseobacterium soli* DSM 19298^T and *Chryseobacterium soldanellicola* KCTC 12382^T

Characteristic	UMB34^T	12483$^{T\ a}$	19298$^{T\ b}$	12382$^{T\ c}$
Cell size (l × w, μm)	0.6–1.2 × 0.4–0.5	0.8–2.0 × 0.4–0.5	1.5–3.0 × 0.6–0.8	ND
Growth range (°C)	1–37	5–37	5–30	5–37
Growth on:				
MacConkey agar	−	−	+	−
Enzyme activity:				
Urease	−	−	+	−
Tween 80 hydrolysis	+	+^{d}	+	−^{d}
Assimilation of:				
L-arabinose	−	+^{d}	−	w
D-mannose	−	+^{d}	+	w
DNA G + C Mol %	39.6–41.6	38.5	39.9	28.8

w weak

[a] Data from Kim et al. (2008)

[b] Data from Weon et al. (2008)

[c] Data from Park et al. (2006)

[d] Used as sole carbon source (GN2 microplate)

temperatures well below their supposed "optimum" growth temperature (Margesin 2009).

UMB34^T did not grow anaerobically in a GasPak pouch although it did form isolated colonies on TSA and R2A under microaerophilic conditions within 2 days at 30°C suggesting that the strain tolerates low oxygen and high CO_2. In the genus description, *Chryseobacterium* species are described as aerobic chemoorganotrophs, but there are exceptions. For example, *Chryseobacterium arothri*, proposed as a synonym of *Chryseobacterium hominis* (Kämpfer et al. 2009b), isolated from fish kidneys, is a facultative anaerobe (Campbell et al. 2008) and the metabolism of C. *scophthalmum* is respiratory and fermentative (Mudarris et al. 1994). *Chryseobacterium* (*Sejongia*) *antarctica*, *Chryseobacterium jeonii*, and *Chryseobacterium marina* are classified as aerobes but are able to grow under microaerophilic conditions and weakly in an anaerobic environment (Yi et al. 2005; Lee et al. 2007). Other close relatives can grow in a CO_2 enriched environment including the aerobe *Elizabethkingia miricola*. *Riemerella* species, at least during primary isolation, need 5–10% CO_2 and are microaerophiles (Bernardet et al. 2006; Vandamme et al. 2006).

UMB34^T assimilated glucose and maltose but not the other substrates present on the API 20 NE strip including L-arabinose, D-mannose, D-mannitol, *N*-acetyl glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid. The inability to utilize D-mannose and L-arabinose distinguishes it from some of its close relatives (Table 1). The strain did produce acid from D-glucose, D-fructose, D-maltose, D-trehalose, starch, glycogen, gentibiose, amygladin, D-mannose (weakly), and D-sucrose (weakly). Production of acid aerobically from glucose and maltose was confirmed in oxidation/fermentation medium, although the color change only was observed after incubation for a few weeks. The isolate hydrolyzed gelatin, casein (skim milk), esculin, Tween 80 and starch, but did not hydrolyze X-Gal, 4-nitrophenyl-β-D-galactopyranoside (PNPG), or urea. It did not reduce nitrate, produce indole, lecithinase or arginine dihydrolase. UMB34^T did not grow on cetrimide or MacConkey agar or on agar with 5, 7, 12, 20% NaCl. UMB34^T did grow on R2A agar with 2% NaCl and on Marine agar as is characteristic for the genus. Some of the physiological characteristics of UMB34^T that differentiate it from its closest relatives are presented in Table 1.

UMB34^T was sensitive, as indicated by zones of inhibition, to the antibiotics tested except for bacitracin and streptomycin. In contrast, the description of the *Chryseobacterium* genus includes resistance to many antimicrobials as a distinctive characteristic (Vandamme et al. 1994). Resistance specifically to penicillin-G was listed as a feature that may differentiate the *Chryseobacterium* genus from some other genera in the *Flavobacteriaceae* family (Bernardet and Nakagawa 2006). For example, closely related species *C. balustinum*, *C. indoltheticum*, and *C. scophthalmum* are resistant to penicillin. Moreover, in a study of *Chryseobacterium* reference strains and isolates from aquatic habitats and fish, 97% were highly resistant to ampicillin, and 3% were moderately resistant or susceptible

Table 2 Comparison of the fatty acid composition (%) of strain UMB34^T with *Chryseobacterium aquaticum* KCTC 12483^T, *Chryseobacterium soldanellicola* KCTC 12382^T, and *Chryseobacterium soli* DSM 19298^T

Fatty acid	UMB34^T	12483^T	12382^T	19298^T
C15:0 iso	30.4	36.4	37.9	37.5
C15:0 anteiso	2.5	1.9	1.0	2.6
C16:1 $\omega7c/\omega6c$[a]	8.4	10.2	ND	ND
C16:1 $\omega6c/\omega7c$[a]	9.3	6.8	12.9	12.1
C16:0	6.6	7.7	0.9	1.3
C15:0 iso 3-OH	2.2	2.0	3.0	3.0
C17:1 iso $\omega9c$	8.5	5.2	18.0	15.0
C17:0 iso	2.4	1.1	0.6	1.3
C16:0 3-OH	3.3	6.3	1.6	1.6
C17:0 iso 3-OH	19.0	15.0	19.7	22.2

Fatty acids comprising less than 2% of total in all strains are not shown

ND not detected

[a] Summed feature 3 comprises C16:1 $\omega7c$ and/or C16:1 $\omega6c$

(Michel et al. 2005). *Chryseobacterium molle*, isolated from a beer bottling plant (Herzog et al. 2008), was sensitive to the tested antibiotics including ampicillin.

The major fatty acids of UMB34^T cultured on TSA at 28°C for 2 days were C15:0 iso, C17:0 iso 3-OH, and summed feature 3 (C16:1 ω7c and/or 16:1 ω6c) (Table 2). The predominance of these acids is characteristic of many species in this genus. In addition, the fatty acid composition is comparable to that of *C. aquaticum* and differs from *C. soldanellicola* and *C. soli* especially in the much lower amount of C17:1 ω9c, recently re-identified as C17:1 iso cis9 by Herzog et al. (2008). An environmental *Chryseobacterium* isolate FRGDSA 480/97, closely related to the three isolates similarly has significantly less of this unsaturated acid (Bernardet et al. 2005).

Genotypic characteristics

The phylogenetic relationships of the three isolates to all validly named species in the *Chryseobacterium* genus and other closely related genera in the *Flavobacteriaceae* family are shown in Fig. 2. UMB10, UMB14, and UMB34^T clustered with one other validated member of the genus, *C. aquaticum* (99.3%) isolated from a water reservoir (Kim et al. 2008) and a *Chryseobacterium* isolate FRGDSA 480/97 (99.1%) from Siberian sturgeon fry (Bernardet et al. 2005). The other closest validly named relatives were *C. soli* (97.1%) isolated from soil (Weon et al. 2008) and in an adjoining cluster *C. soldanellicola* (96.9%), *C. scophthalmum* (96.6%), *C. indoltheticum* (96.6%), *C. balustinum* (96.4%), and *C. piscium* (95.5%) isolated from fish or marine associated habitats. Whether or not they originated from aeolian deposition or underlying permafrost could not be determined. It is worth noting, however, that strains UMB10, UMB14 and UMB34^T trapped in the basal glacier ice for at least 120,000 years are most closely related to species isolated from fish or water.

To elucidate the taxonomic status of the proposed species *C. greenlandense,* DNA–DNA hybridization experiments were done. The results showed low genome homology between UMB34^T and *C. aquaticum* and *C. soldanellicola* (19–30%). The level of relatedness of *C. aquaticum* to *C. soldanellicola* was 19%, which is within the range of 15–39% reported by Kim et al. (2008) for *C. aquaticum* to *C. indoltheticum*, *C. scophthalmum*, and *C. soldanellicola*. The homology between UMB34^T and *C. soli* and *C. jejuense* was higher (45–56%) but well below the accepted value of 70%. These DNA–DNA hybridization data are substantiated by the results from the comparative genomic fingerprinting procedure that was done using the ERIC primer. The nearly identical banding patterns of UMB10, UMB14 and UMB34^T were distinct from their closest validly named relative, *C. aquaticum*, and from *C. soldanellicola, C. soli* and *C. jejuense* indicating low genome homology between UMB34^T and its close phylogenetic relatives (Fig. 3).

The G + C mol% of UMB34^T was calculated to be 39.6–41.6, which is higher than the reported G + C contents of most of the closely related species (see Table 1). Typically, the G + C contents of *Chryseobacterium* spp. range between 33 and 38 mol% but two new *Chryseobacterium* species, *C. soli* and *C. jejuenese*, had G + C contents of 39.9 and 41.4, respectively (Weon et al. 2008).

Inclusion of UMB10, UMB14 and UMB34^T into the *Chryseobacterium* genus is based on the 16S rRNA genes phylogenetic analysis in conjunction with the fatty acid composition, presence of flexirubin pigment, acid production from carbohydrates, production of proteolytic enzymes, and the ability to grow on marine agar. The distinctive environment from which the strains were isolated, the presence of unique morphological features and the small cell size distinguishes these novel isolates from all other *Chryseobacterium* species with validly described names. The sensitivity to ampicillin also distinguishes the isolates from most *Chryseobacterium* species and its higher G + C content and inability to utilize mannose differentiates this proposed species from its closest validly named relatives. Moreover, the different ERIC-PCR genomic fingerprinting profiles and the low genomic DNA–DNA homology between UMB34^T and close validly named relatives, *C. aquaticum* and *C. soldanellicola,* indicate that UMB10, UMB14 and UMB34^T represent a new species belonging to the *Chryseobacterium* genus for which we propose the name, *Chryseobacterium greenlandense*.

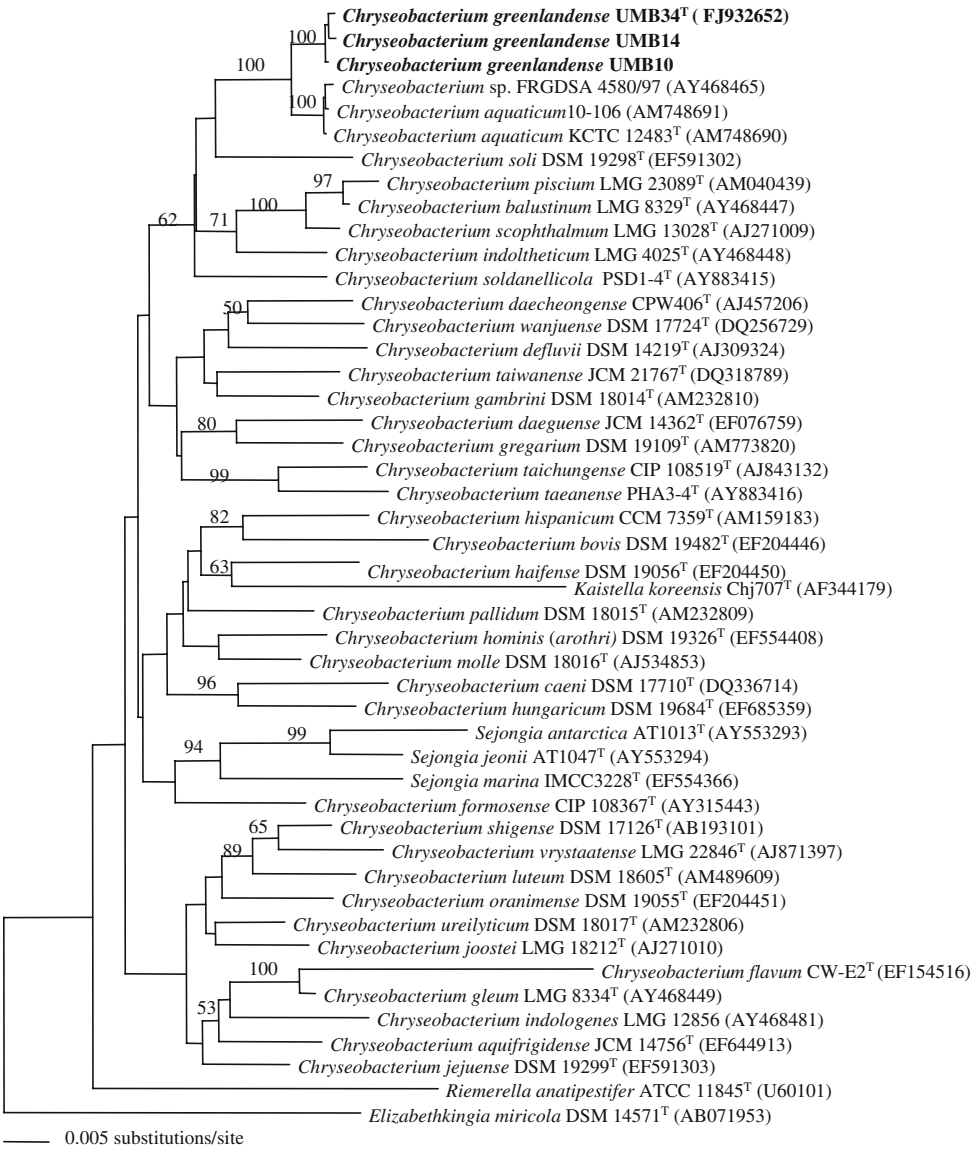

Fig. 2 Phylogenetic relationships of *Chryseobacterium greenlandense* sp. nov. with all validly named species from the genus *Chryseobacterium* and other genera from the family *Flavobacteriaceae*. The tree is based on distance matrix analysis, neighbor-joining algorithm with Jukes-Cantor model. Bootstrap values (greater than 50%) generated from 10,000 replicates are shown above the nodes

Description of *Chryseobacterium greenlandense* sp. nov

Chryseobacterium greenlandense (green.lan.den'se N.L. neuter. adj., *greenlandense* of Greenland, where the novel species was isolated from). Colonies are round, convex, yellow to dark orange, 1–2 mm in diameter after incubation for 2–3 days at 25°C. Colony diameter increases and edges may become rhizoid after a few weeks of incubation, especially on low nutrient medium such as R2A. Cells are short rods, 0.6–1.2 μm × 0.4–0.5 μm with an average volume of 0.08 μm^3. Cells stain Gram-negative, have buds and small protrusions or blebs. The cells have no flagella although they exhibit a swarming-like behavior. Cells produce a flexirubin type pigment that becomes brown when 20% KOH is added. Cells are catalase and oxidase positive. Growth range is 1–37°C and the optimal growth rate is at 30°C, with a doubling time of 2 h in TSB without added dextrose. There is no growth at 42°C. Growth occurs on marine but not on MaConkey or cetrimide agars. There is growth on media containing 2% but not 5% NaCl. Acid is produced from sugars including D-glucose, D-fructose, D-maltose, D-trehalose, starch, glycogen, gentibiose, amygladin, D-mannose (weakly), D-sucrose (weakly). Glucose and maltose are assimilated but L-arabinose, D-mannose, D-mannitol, *N*-acetyl glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized. Skim milk (casein), gelatin, Tween 80, starch and esculin are hydrolyzed but PNPG, X-Gal, lecithin, and urea are not. Nitrate is not reduced, indole and arginine dihydrolase are not produced. Major fatty acids are C:15:0 iso, C:17:0 iso 3-OH and summed feature 3 (C16:1 ω7c and/or 16:1 ω6c). Sensitive to ampicillin, ciprofloxacin, erythromycin, nalidixic acid, neomycin, penicillin, rifampicin, and vancomycin.

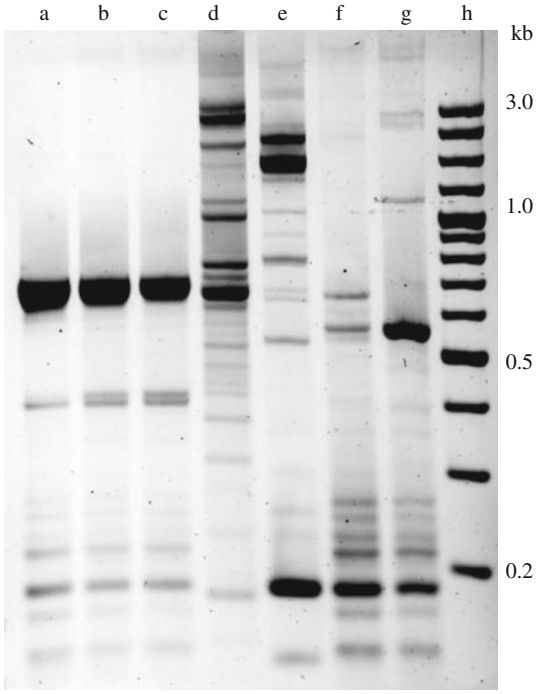

Fig. 3 Comparative ERIC-PCR genomic fingerprinting profiles of UMB34^T (*lane a*), UMB10 (*lane b*), UMB14 (*lane c*) *Chryseobacterium aquaticum* (*lane d*), *Chryseobacterium soldanellicola* (*lane e*), *Chryseobacterium soli* (*lane f*) and *Chryseobacterium jejuense* (*lane g*) are shown. Molecular weight marker is in *lane h*

Resistant to streptomycin and bacitracin. G + C is 39.6–41.6 mol%.

The type strain, UMB 34^T (=CIP 110007T = NRRL B-59357), was isolated from a 3,043 m deep, 120,000-year-old Greenland glacier ice core sample after a low temperature enrichment procedure.

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