Jannaschia helgolandensis gen. nov., sp. nov., a novel abundant member of the marine *Roseobacter* clade from the North Sea

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Heterotrophic bacteria were isolated from a water sample taken from the North Sea, 2 km off the coast of the island of Helgoland, by direct plating of the serially diluted sample on complex marine media. Sixteen of 80 strains from the highest sample dilution belonged to the '*Roseobacter–Sulfitobacter–Silicibacter*' group within the α -subclass of the *Proteobacteria* on the basis of partial 16S rDNA sequence analysis. Phylogenetic analysis of nearly complete 16S rDNA sequences showed that the closest relative of two strains, Hel 10^T and Hel 26, was *Ketogulonicigenium vulgare* (94.4 % similarity). These strains were Gram-negative, non-motile rods, obligate aerobes, required sodium ions and 1–7 % sea salts for growth and did not produce bacteriochlorophyll. Their optimal growth temperature was 25–30 °C. The strains had Q-10 as the dominant respiratory quinone. Chemotaxonomic analysis showed a combination of ester-linked 3-OH 10 : 0, 12 : 1 and amide-linked 3-oxo 14 : 0 (or 3-OH 14 : 1) and 3-OH 14 : 0 fatty acids, which appears to be a unique feature of strains Hel 10^T and Hel 26 within this subsection of the α -subclass of the *Proteobacteria*. Based on 16S rDNA sequence analysis and chemotaxonomic data, the strains are assigned to a new genus and species, *Jannaschia helgolandensis* gen. nov., sp. nov., with the type strain Hel 10^T (= DSM 14858^T = NCIMB 13941^T).

INTRODUCTION

While the majority of marine bacteria detected by molecular analyses have not yet been cultivated on microbiological media using classical techniques, the '*Roseobacter–Sulfitobacter–Silicibacter*' group of the α -subclass of the *Proteobacteria* is an exception (Eilers *et al.*, 2000; Rappé *et al.*, 2000). Small-subunit ribosomal sequences falling into this group are frequently found in marine clone libraries and have also been shown to be abundant in marine bacterioplankton communities using ribosomal probes (Gonzalez & Moran, 1997; Brinkmeyer *et al.*, 2000; Weidner *et al.*, 2000; Zubkov *et al.*, 2001a). At the same time, a large number of metabolically diverse strains from

the '*Roseobacter–Sulfitobacter–Silicibacter*' group have been cultivated over the last few years. Strains belonging to the species *Roseobacter denitrificans* and *Roseobacter litoralis* are strict aerobes that are capable of photosynthesis using bacteriochlorophyll *a* (Shiba, 1991). Other genera of aerobic phototrophs (members of the genera *Erythromicrobium*, *Erythroacter* and *Porphyrobacter* or *Roseibium*) were found to belong to different subgroups within the α -subclass of the *Proteobacteria* (Yurkov *et al.*, 1994; Yurkov & Beatty, 1998a; Suzuki *et al.*, 2000). Aerobic heterotrophs from the '*Roseobacter–Sulfitobacter–Silicibacter*' group also include members of the genera *Octadecabacter* (Gosink *et al.*, 1997), *Sulfitobacter* (Sorokin, 1995; Pukall *et al.*, 1999b; Labrenz *et al.*, 2000), *Sagittula* (Gonzalez *et al.*, 1997), '*Marinosulfonomonas*' (Holmes *et al.*, 1997), *Antarctobacter* (Labrenz *et al.*, 1998), *Roseovarius* (Labrenz *et al.*, 1999), *Ruegeria* (Uchino *et al.*, 1998), *Ketogulonicigenium* (Urbance *et al.*, 2001), *Roseivivax* (Suzuki *et al.*, 1999), *Silicibacter* (Petursdottir & Kristjansson, 1997) and *Staleyia* (Labrenz *et al.*, 2000). Most strains of the genera *Roseovarius*, *Staleyia* and *Roseivivax*, like *Roseobacter denitrificans* and *Roseobacter litoralis*, contain bacteriochlorophyll *a*. The physiological characteristics of described species within these genera are

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Two-dimensional thin-layer chromatograms of polar lipids of strains Hel 10^T and Hel 26 are available as supplementary material in IJSEM Online.

diverse, ranging from the degradation of lignin (Gonzalez *et al.*, 1997) and other aromatic compounds (Buchan *et al.*, 2000) to oxidation of sulfite (Sorokin, 1995; Pukall *et al.*, 1999b; Labrenz *et al.*, 2000) and growth on methanesulfonic acid (Holmes *et al.*, 1997). The abundance of strains from the 'Roseobacter-Sulfitobacter-Silicibacter' group has been correlated with algal blooms and the occurrence of large amounts of dimethylsulfoniopropionate, suggesting their participation in sulfur cycling (Gonzalez *et al.*, 1999, 2000; Ansedé *et al.*, 2001; Zubkov *et al.*, 2001b). Strains belonging to the 'Roseobacter-Sulfitobacter-Silicibacter' group have been isolated from various environments including marine bacterioplankton, both coastal areas and the open ocean, from Antarctica (Gosink *et al.*, 1997; Labrenz *et al.*, 2000; Söller *et al.*, 2000), hypersaline environments (Labrenz *et al.*, 1999, 2000) and black smokers (Yurkov & Beatty, 1998b). Some strains are suspected of being specifically associated with marine invertebrates (Ruiz-Ponte *et al.*, 1998, 1999; Boettcher *et al.*, 2000; Grigioni *et al.*, 2000; Barbieri *et al.*, 2001) or can colonize surfaces rapidly (Lafay *et al.*, 1995; Prokic *et al.*, 1998; Dang & Lovell, 2000). There are symbioses between marine algae and 'Roseobacter-Sulfitobacter-Silicibacter' group species where molecular evidence for co-evolution has been described (Ashen & Goff, 2000).

However, those strains of the 'Roseobacter-Sulfitobacter-Silicibacter' group that are readily culturable do not appear to show identical 16S rRNA sequences to the numerically abundant bacterioplankton strains (Eilers *et al.*, 2001). Given the biochemical and physiological heterogeneity within this group, it is difficult to predict the precise biogeochemical roles of these currently uncultivated bacteria. Similarly, the ecological significance of the peculiar type of aerobic anoxygenic photosynthesis in isolated strains of some genera (Yurkov & Beatty, 1998a; Candela *et al.*, 2001) and the reasons for their apparent success in marine bacterioplankton communities remain topics for further research.

In an attempt to characterize members of the 'Roseobacter-Sulfitobacter-Silicibacter' group further, we isolated a large number of metabolically and phylogenetically diverse marine strains from the North Sea by using different cultivation approaches and identified members of this group by means of 16S rDNA-based screening techniques. Enrichments made from filtered (5 µm) sea water with a variety of media and incubation conditions yielded 84 members of the α -subclass of the *Proteobacteria* from a total of 410 strains, as judged by specific 16S rDNA-targeted multiplex PCR (signature PCR) and fluorescent *in-situ* hybridization analysis (Uphoff *et al.*, 2001). Of these strains, eight were multiple isolates, based on genomic fingerprints (RAPD PCR). Fourteen of the remaining 76 different strains belonged to the 'Roseobacter-Sulfitobacter-Silicibacter' group on the basis of partial 16S rDNA sequencing. Among 80 isolates cultivated directly from the highest dilution of fresh unfiltered sea-water samples on complex marine media and

screened in a similar way, they accounted for 16 different strains. Two isolates from the highest serial dilution of North Sea water (Hel 10^T, Hel 26) could not be assigned to a described genus on the basis of almost-complete 16S rDNA sequences and are therefore described in this paper.

METHODS

Isolation of strains. A water sample was taken from a depth of 10 m using a Ruttner sampler at the Tiefe Rinne, about 2 km off the shore of the island of Helgoland in the North Sea (54°08'N, 7°52'E) on 23 September 1998. The water temperature was 15.5 °C, oxygen content was 8.1 mg l⁻¹ and Secchi disc visibility was 5.5 m. The sample was transported immediately to the laboratory and processed. An aliquot (100 µl) was serially diluted (10⁻¹, 10⁻², 10⁻³) in sterile-filtered (0.2 µm), autoclaved sea water. Subsamples (50 µl) were spread on agar plates with medium DSMZ 172 [l⁻¹: 1.0 g yeast extract (Difco), 1.0 g tryptone (Difco), 24.7 g NaCl, 0.7 g KCl, 6.3 g MgSO₄ · 7H₂O, 4.6 g MgCl₂ · 6H₂O, 1.2 g CaCl₂ · 2H₂O, 0.2 g NaHCO₃, 15 g agar (Difco)] containing 0.002% cycloheximide. Colonies from the highest dilution were picked and restreaked several times for purification.

Culture conditions. After isolation and purification, the strains under investigation were grown routinely on LB agar containing additional sea salts (LBSS) (l⁻¹: 10.0 g tryptone, 5.0 g yeast extract, 10.0 g NaCl, 14.0 g sea salts (Sigma), 15 g agar). Use of this medium resulted in better growth than the original isolation medium. Preservation of the isolates was performed in glycerol as follows. Five millilitres LBSS broth was inoculated with a loopful of cell material and incubated for 2–3 days at 30 °C with shaking. Aliquots of 1.5 ml of the suspension were centrifuged at 7000 g for 5 min and the supernatant was discarded. After resuspending the pellet in 500 µl fresh LBSS broth, 750 µl sterile glycerol (99.5%) was added and mixed well. The suspension was then equilibrated on ice for 30 min, followed by freezing at -18 °C for 2 h and final storage at -70 °C. For reactivation, 50 µl suspension was streak-plated on LBSS agar. Long-term storage was carried out at the DSMZ by freeze-drying and storage in liquid nitrogen.

Determination of physiological characteristics. A loopful of cell material of strains Hel 10^T and Hel 26 was taken from a fresh culture on solid LBSS medium. For each of the strains, a suspension corresponding to McFarland standard 1 (OD₅₅₀ = 0.25; bioMérieux) was prepared in 10 ml saline buffer supplemented with 2% (w/v) sea salts (Sigma). The OD₅₅₀ was adjusted by addition of buffer or cell material as appropriate. One drop of this suspension was added to each of the test tubes or test plates. Incubation of the tests was performed at 25 °C.

Temperature range for growth was tested in LBSS broth from 4 to 60 °C and halotolerance was tested in medium devoid of NaCl with 0, 1, 3, 5, 7, 10, 13 and 15% (w/v) sea salts added. The pH range for growth was determined in the range 5.0–11.0 in steps of one pH unit. The pH value was adjusted by addition of HCl or NaOH. These tests were set up in duplicate. Capacity for anaerobic growth was also tested on LBSS medium incubated in an anaerobic jar.

The following physiological tests were carried out according to Gordon *et al.* (1973): catalase reaction, oxidase reaction, presence of urease, decomposition of Tween 80, starch hydrolysis and nitrite production.

Gelatin liquefaction was tested after Gerhardt *et al.* (1981) in that plates of LBSS containing 0.4% gelatin were incubated with the strains for 7 and 14 days. Plates were then flooded with warm (55 °C) 0.5 M sulfuric acid, saturated with Na₂SO₄. A resulting clear circular zone

around the colony indicated digestion of the gelatin. Hydrolysis of aesculin was tested according to Lányi (1987) in a medium consisting of (l^{-1}) 10.0 g Bacto-peptone, 1.0 g sodium citrate, 1.0 g aesculin and 0.05 g iron citrate at pH 6.8–7.0.

Carbon utilization was tested in standard mineral base medium (Stanier *et al.*, 1966) containing 0.2 % of the carbon source. A negative control without carbon source was also included. As no growth could be observed in any of these tests, they were repeated with the addition of three drops of sterile 0.1 % yeast extract to each of the test tubes. Even under these conditions, the negative control did not show any growth. The tests were examined for growth daily for up to 2 weeks until no further growth was observed in the test tubes. Carbon sources tested in this way were glucose, acetate, propionate, butyrate, pyruvate, DL-lactate, L-aspartate, asparagine, L-glutamate, L-proline, L-serine, DL-alanine, L(+) -ornithine, succinate and methanol.

Physiological reactions were also tested using the substrate panels of the API 20 NE and API 50 CH systems (bioMérieux). Additional carbon sources covered by these systems were D-arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate, phenylacetate, glycerol, erythritol, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -xyloside, galactose, D-fructose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, β -gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate and 5-ketogluconate.

Reduction of nitrate, indole production, fermentation of D-glucose, presence of arginine dihydrolase, urease and β -galactosidase and hydrolysis of aesculin and gelatin were additionally tested by reading and interpreting the corresponding API tests.

Microscopic investigations. Primary morphological characterization was done by light microscopy, including phase-contrast observations. The size and ultrastructure of the cells were determined by electron microscopy. Cell morphology was investigated using slides covered with 2 % (w/v) agar (dissolved in water). Transmission electron microscope investigations were carried out as described previously (Rheims *et al.*, 1999).

Chemotaxonomy. Analysis of fatty acid methyl esters was performed with 20 mg freeze-dried biomass as described previously (Labrenz *et al.*, 1998). Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried material using a two-stage extraction method and analysed as described previously (Tindall, 1990a, b).

Determination of base composition of DNA. Isolation of DNA (Cashion *et al.*, 1977) and determination of the DNA G + C content by HPLC (Mesbah *et al.*, 1989) followed described procedures.

DNA–DNA hybridization. DNA–DNA similarity studies were performed by the renaturation method (Escara & Hutton, 1980; Huß *et al.*, 1983). Similarity values were calculated according to the methods of Jahnke (1992).

Phylogenetic inference. Genomic DNA was extracted from bacterial cells and purified using a protocol described previously (Pukall *et al.*, 1998). The primer pair 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1527r (5'-AGAAAGGAGGTGATCCAGCC-3') was used for amplification of the 16S rRNA gene (Lane, 1991). PCR amplification of 16S rDNA by PCR was done as described by Pukall *et al.* (1999a). Analysis of the 16S rDNA sequences obtained from isolates Hel 10^T and Hel 26 followed the method described by Rainey *et al.*, (1996) using the Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) and an Applied Biosystems model 373A automated

DNA sequencer. Sequences were aligned manually and compared with sequences published previously. These were stored in the DSMZ internal database, consisting of more than 6000 16S rDNA sequence entries, including those from the Ribosomal Database Project (Maidak *et al.*, 2001) and EMBL. Similarity values were transformed into genetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) and the algorithm of DeSoete (1983) were used in the construction of the phylogenetic dendrogram. All analyses were done on a SUN SparcII workstation.

RESULTS AND DISCUSSION

Occurrence

Approximately 20 % of isolates obtained from a North Sea water sample and from enrichments made from filtered (5 μ m) North Sea water using a diverse spectrum of incubation conditions (Uphoff *et al.*, 2001) belonged to the 'Roseobacter–Sulfitobacter–Silicibacter' group on the basis of partial 16S rDNA sequencing. Strains Hel 10^T and Hel 26 were isolated by direct plating of serially diluted sea-water samples on complex media. They can therefore be assumed to have represented a major component of the cultivable bacteria of North Sea bacterioplankton at the time of isolation.

Colony and cell morphology

Strains Hel 10^T and Hel 26 developed whitish, shiny colonies on agar media. The individual colonies remained rather small, even after prolonged incubation. Cells were irregular rods with a length of 1.9–3.2 μ m and a width of 0.7–1.1 μ m (Fig. 1). Both strains could be shown to stain Gram-negatively by classical staining techniques as well as by cell lysis after the addition of 3 % (w/v) KOH. As judged by microscopic investigations, the two strains did not form spores. Cells adhering together to form chains were frequently observed. White inclusion bodies were often present, which were clearly not gas vesicles, as judged by transmission electron microscope investigations (Fig. 1b and data not shown).

Physiological characteristics

Both isolates grew strictly aerobically. Tests for the presence of cytochrome oxidase were performed repeatedly with fresh reagent and cultures of different ages. In all cases, Hel 10^T and Hel 26 showed a weakly positive reaction. Neither strain reduced nitrate to nitrite.

Growth was poor at 15 °C, ranging to 30 °C, with an optimum temperature of 25–30 °C. The pH range tolerated for growth was 7.0–8.0. In media devoid of sea salts, no growth of either isolate was observed. When only sodium chloride was added to the test medium, the isolates also failed to grow. Therefore, determination of halotolerance was carried out with the addition of commercially available sea salts; the growth range was 1–7 % (w/v).

Fig. 1. Light microscope (a; bar, 5 μ m) and transmission electron microscope (b; bar, 1 μ m) images of cells of strain Hel 26. Observations made were found to be representative for Hel 10^T as well.

Carbon-source utilization in 5 ml standard mineral base medium (Stanier *et al.*, 1966) containing 0.2 % of the carbon source and three drops of 0.1 % yeast extract showed utilization of D-glucose, DL-lactate, succinate and (for Hel 26 only) L-aspartate. Carbon-source utilization tests in API test medium showed growth for mannose, mannitol, gluconate, malate, citrate, glycerol, ribose, D-xylose, galactose, D-fructose, rhamnose, inositol, sorbitol, cellobiose, D-arabitol, 2-ketogluconate and (for Hel 26 only) D-fucose and 5-ketogluconate. Three substrates (L-aspartate, D-fucose, 5-ketogluconate) were therefore used by strain Hel 26 but not by strain Hel 10^T. Further carbon sources that were tested but gave negative results are listed in Methods.

Phylogenetic inference

Phylogenetic inferences were based on the analysis of nearly complete 16S rDNA sequences of strains Hel 10^T and Hel 26. The 16S rDNA sequences of strains Hel 10^T and Hel 26 were identical. Phylogenetic analysis revealed that the strains were members of the α -subclass of the *Proteobacteria* and were associated with the 'Roseobacter-Sulfitobacter-Silicibacter' group. Their nearest phylogenetic neighbours were *Ketogulonicigenium vulgare* (94.4 % similarity) and the currently unclassified strains CV919-312 (Boettcher *et al.*, 2000) and SFR3 (L. Giuliano, M. DeDomenico, E. DeDomenico, M. Höfle and M. M. Yakimov, unpublished accession no. AJ002565), showing respective similarity values of 95.3 and 94.3 %. A dendrogram of 16S rDNA relationships, displaying the positions of strains Hel 10^T and Hel 26 to their closest neighbours, is shown in Fig. 2. The dendrogram shows that the Hel strains are phylogenetically distant from the genus *Ketogulonicigenium*, forming a distinct subline. Although the 16S rDNA sequences of strains Hel 10^T and Hel 26 were identical, the two strains differed physiologically in the utilization of L-aspartate, D-fucose and 5-ketogluconate as carbon sources. Determination of DNA relatedness between Hel 10^T and Hel 26 by the spectrophotometric method revealed that those two

isolates could not be distinguished and therefore belong to the same species. Due to the differences in their physiological characteristics, the isolates may represent different strains of the same species.

The G + C content was determined to be 63.0 mol% for strain Hel 10^T and 63.1 mol% for strain Hel 26.

Fig. 2. Dendrogram of 16S rDNA relatedness showing the positions of strain Hel 10^T and Hel 26 next to their nearest identified neighbours within the α -subclass of the class *Proteobacteria*. The tree was calculated by the neighbour-joining method from almost-complete sequences. Percentages of 1000 bootstrap resamplings that support branching points above 65 % confidence are indicated. Bar, 5 nucleotide substitutions per 100 sequence positions. The tree was rooted with *Escherichia coli* as an outgroup. Sequence accession numbers are given in parentheses.

Chemotaxonomic properties

Analysis of the respiratory quinone composition of strains Hel 10^T and Hel 26 indicated that Q-10 predominated in both strains. The fatty acid compositions of the two strains were essentially identical, with 18:1 ω 7c predominating. The remaining fatty acids are listed in Table 1.

The polar lipid compositions of the two strains were virtually identical, with the phospholipids comprising phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine, with an aminolipid also being present (two-dimensional thin-layer chromatograms are available as supplementary material in IJSEM Online).

The presence of Q-10 as the dominant respiratory quinone is a feature of many, but not all, members of the α -subclass of the *Proteobacteria*. It is also found in *Legionella* species (γ -*Proteobacteria*), but in this genus it is not the sole major component. In addition to the presence of Q-10 as the major respiratory lipoquinone, the polar lipid composition and fatty acid composition of Hel 10^T and Hel 26 are indicators of the fact that these two strains are members of a subgroup within the α -subclass of the *Proteobacteria*. Although it may not be possible to determine with absolute certainty the branching order within the group defined by members of the genera '*Roseobacter*–*Sulfitobacter*–*Silicibacter*' based on 16S rDNA sequences, and the branching order of this group with strains Hel 10^T and Hel 26, *Rhodobacter veldkampii* and members of the genus *Ketogulonicigenium* may also not be unambiguous, it is sufficient that groups be clearly distinguished from one another (Tindall, 1994), there being a distinct and subtle difference between phyletic lineages and phyletic groups (Gilmour, 1940).

Table 1. Fatty acid compositions of strains Hel 10^T and Hel 26

Values are percentages of total fatty acids.

Fatty acid	Ester-linked		Ester- and amide-linked	
	Hel 26	Hel 10 ^T	Hel 26	Hel 10 ^T
3-OH 10:0	5.0	5.6	3.7	4.5
12:1	4.2	4.9	3.0	3.4
3-OH 14:1/ 3-oxo 14:0	0.0	0.0	1.88*	2.12*
3-OH 14:0	0.0	0.0	1.12*	1.36*
17:0	1.9	1.0	1.8	1.0
18:1 ω 7c	52.9	45.1	52.7	45.0
18:0	8.6	10.8	9.3	11.7
Methyl 18:1	2.9	5.7	4.0	7.6
Unknown	1.1	0.9	0.0	0.0
Cyclo 19:0	21.7	25.1	20.6	22.4
20:0	1.7	0.9	2.1	1.0

*Presumptive amide-linked fatty acids.

It is particularly interesting that strains Hel 10^T and Hel 26 synthesize 17:0, 3-OH 10:0, 3-OH 14:0 and a 12:1 fatty acid in addition to 18:1 ω 7c, 18:0 and cyclo 19:0. In addition, a fatty acid was present that could not be identified unambiguously as either 3-oxo-14:0 or 3-OH-14:1. In the case of the 3-OH and 3-oxo fatty acids, 3-OH 10:0 was evidently ester-linked, whereas the 3-oxo 14:0 (or 3-OH 14:1) and 3-OH 14:0 fatty acids appeared to be amide-linked. The combination of ester-linked 3-OH 10:0, 12:1 and amide-linked 3-oxo 14:0 (or 3-OH 14:1) and 3-OH 14:0 fatty acids appears to be a unique feature of strains Hel 10^T and Hel 26 within this subsection of the α -subclass of the *Proteobacteria* (for the fatty acids in other genera, see Labrenz *et al.*, 1998, 1999, 2000; Urbance *et al.*, 2001). Fatty acid heterogeneity has also been shown within the '*Roseobacter*–*Sulfitobacter*–*Silicibacter*' group, reflecting differences between or even within genera (i.e. the genus *Ruegeria*), where such differences correlate with 16S rDNA sequence heterogeneity, suggesting that further taxonomic work is necessary. Fewer studies include the polar lipid composition of members of this group, but those results available also indicate that there is some degree of heterogeneity, which would also be useful in further delineating the various genera.

In the case of strains Hel 10^T and Hel 26, we interpret the chemical composition of the cell as being indicative of the fact that these two strains, which obviously represent a single species, should also be placed in a new genus.

Description of *Jannaschia* gen. nov.

Jannaschia (Jan.na'schi.a. N.L. fem. n. *Jannaschia* after the German microbiologist Holger W. Jannasch, one of the pioneers of marine microbiology, who had a tremendous impact on the field and was particularly devoted to studying microbial growth kinetics in sea water and the microbial ecology of hydrothermal vents).

Gram-negative, irregular rods with a tendency to form chains during growth. On LBSS agar, strains develop small, whitish, shiny colonies within 3–5 days. They do not form spores. Growth is poor at 15 °C and optimal at 30 °C. The pH tolerance for growth is 7.0–8.0. In media devoid of salts or containing only sodium chloride, no growth is observed. Growth is observed with 1–7 % (w/v) sea salts. Weakly positive reaction in tests for cytochrome oxidase. No strains reduce nitrate to nitrite. Strictly aerobic, non-fermentative heterotrophs. The predominant respiratory quinone is ubiquinone 10. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and an aminolipid. The major fatty acids are 18:1 ω 7c, 18:0, cyclo 19:0, 17:0, 3-OH 10:0, 3-OH 14:0, 3-oxo-14:0/3-OH 14:1 and 12:1. Among the hydroxylated fatty acids, 3-oxo-14:0/3-OH 14:1 and 3-OH 14:0 appear to be amide-linked, whereas 3-OH 10:0 appears to be ester-linked. The type species is *Jannaschia helgolandensis*.

Description of *Jannaschia helgolandensis* sp. nov.

Jannaschia helgolandensis (hel.go.lan.den'sis. N.L. fem. adj. *helgolandensis* of the island of Helgoland, where the type strain was isolated).

In addition to the properties described for the genus, the following properties are observed. Gram-negative, non-spore-forming, strictly aerobic and heterotrophic bacteria. Cells are non-motile, irregular rods, 1.9–3.2 µm long and 0.7–1.1 µm wide. Dividing cells tend to adhere together, forming chains. White inclusion bodies are observed. Catalase-positive and weakly oxidase-positive. DNA G + C content is 63.0–63.1 mol%. Cells grown on LBSS develop small, whitish, shiny colonies within 3–5 days. Optimal growth occurs at 25–30 °C; slow growth at 15 °C. Hydrolysis of gelatin, starch, Tween 80, urea and aesculin is not observed. Does not reduce nitrate to nitrite. Carbon-source utilization in standard mineral base medium (Stanier *et al.*, 1966) containing 0.2 % of the carbon source and 0.1 % yeast extract shows utilization of D-glucose, DL-lactate, succinate, mannose, mannitol, gluconate, malate, citrate, glycerol, ribose, D-xylose, galactose, D-fructose, rhamnose, inositol, sorbitol, cellobiose, D-arabitol and 2-ketogluconate. Utilization of L-aspartate; D-fucose and 5-ketogluconate is strain-dependent (negative for type strain). Chemotaxonomic properties and other characteristics are as for the genus.

The type strain, strain Hel 10^T (=DSM 14858^T=NCIMB 13941^T), and reference strain Hel 26 were isolated from a sample of sea water obtained at a depth of 10 m off the coast of the island of Helgoland in the North Sea.

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