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Oceanibulbus indolifex, gen. nov., sp. nov., a North Sea Alphaproteobacterium producing bioactive metabolites

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Abbreviations: DMSP, dimethylsulfiopropionate

The EMBL accession number for the 16S rRNA gene sequence of HEL-45^T is AJ550939.

Abstract

A water sample from the North Sea was used to isolate by serial dilution and spread plating the abundant heterotrophic bacteria able to grow on complex marine media. Phylogenetic analysis of nearly complete 16S rRNA genes revealed that one of the strains, HEL-45^T, had 97.4 % sequence similarity to *Sulfitobacter mediterraneus* and 96.5 % sequence similarity to *Staleya guttiformis.* Strain HEL-45^T is a Gram negative, non-motile rod, obligate aerobe and requires sodium and at least 1 up to 7 % sea salts for growth. It contains storage granules and does not produce bacteriochlorophyll. The optimal growth temperature is at 25°C – 30°C. The DNA base composition is 60.1 Mol % G+C. Strain HEL-45^T has Q10 as the dominant respiratory quinone. The major polar lipids are phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidyl choline, phosphatidyl ethanolamine, and an aminolipid. The fatty acids comprise 18:1ω7c, 18:0, 16:1ω7c, 16:0, 3-OH 10:0, 3-OH-12:1 (or 3-oxo 12:0), and traces of an 18:2 fatty acid. Among the hydroxylated fatty acids only the 3-OH 12:1 (or 3-oxo 12:0) appears to be amide linked, whereas the 3-OH 10:0 appears to be ester linked. The minor fatty acid components (between 1-7%) allow three subgroups to be distinguished in the *Sulfitobacter/Staleya* clade placing HEL-45^T into a separate lineage characterized by the presence of 3-OH 12:1 (or 3-oxo 12:0), and both ester- and amide-linked 16:1ω7c phospholipids. HEL-45^T produces indole and derivatives thereof, several cyclic dipeptides and thryptanthrin. Phylogenetic analysis of 16S rRNA gene sequences and chemotaxonomic data support the description of a new genus and species, to include *Oceanibulbus indolifex,* with the type strain HEL-45^T (DSM 14862; NCIMB 13983).

Introduction

In order to characterize the cultivable part of the bacterioplankton of the North Sea, a water sample was investigated which was taken at a distance of 2 km from the island of Helgoland (10 m depth). Samples were immediately serially diluted and spread plated on marine agar media. After 2 weeks of incubation, appr. 100 colonies were picked at random from plates with the highest sample dilution. The isolates can therefore be expected to belong to the more abundant of the cultivable bacteria, which grow under these conditions, at that particular sampling date. Partial sequencing (forward primer F27, about 500 basepairs of sequence) of 16S rRNA genes of the isolates showed that appr. 50 % showed less than 97 % sequence similarity to described species. Sixteen isolates fell into the so-called *Roseobacter* lineage (Eilers et al., 2001), a cluster of genera phylogenetically related to the genus *Roseobacter* within the Alphaproteobacteria. These bacteria have attracted interest over the last few years, because they are the closest cultivated relatives of the second most abundant uncultivated lineage in the marine picoplankton, the SAR83 cluster (Giovannoni & Rappé 2000; Rappé et al., 2000).

Cultivated representatives of the *Roseobacter* lineage display interesting physiological capabilities which may be of great importance for the marine ecosystem, e.g. aerobic anoxygenic photosynthesis (Yurkov & Beatty, 1998a), the turnover of the greenhouse gas DMSP (dimethylsulfoniopropionate) (Zubkov et al. 2001), or the production of sodium-channel blocking toxins, which has been found in *Sulfitobacter* strains from toxic dinoflagellates (Vasquez et al. 2001, 2002).

Of the *Roseobacter* clade strains isolated from the Helgoland water sample, one group of strains (HEL-10^T, HEL-43, HEL-26) was only distantly related to any of the described genera in this group and has recently been described as *Jannaschia helgolandensis* (Wagner-Döbler et al. 2003). Here we report the description of strain HEL-45^T, which is phylogenetically related to both *Sulfitobacter* and *Staleya* and produces a number of interesting secondary metabolites which were analysed by Kampen (2001), Schröder

(2002) and Lurtz et al. (2002) (Fig. 1). They identified indole and several indole derivatives, e.g. indole-3-carboxylic-thiomethylester, 3-indole-carbaldehyde and 3,3-bis-(indol-3-yl)-propane-1,2-diol. Moreover, bioactive compounds were found. Three cyclic dipeptides were identified, namely cyclo-(leu,pro), cyclo-(phe,pro) and cyclo-(tyr,pro) which are known to have weak antiviral, antibiotic and antitumor activity (Milne et al. 1998). In addition, tryptanthrin was found which is known to have activity against some Gram positive bacteria as well as fungi of the genera *Trichophyton* and *Microsporum* (Honda et al. 1979).

Methods

Isolation of strain HEL-45^T. A water sample was taken from 10 m depth using a Ruttner sampler at the “Tiefe Rinne” approx. 2 km offshore the island of Helgoland in the North Sea (54°08`N and 7°52`E) on 23rd September 1998. The water temperature was 15.5 °C, oxygen content 8.1 mg L^{-1} and Secchi disc visibility was 5.5 m. The sample was immediately transported 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 DSMZ medium 172 (1.0 g yeast extract (Difco), 1.0 g tryptone (Difco), 24.7 g NaCl, 0.7 g KCl, 6.3 g $MgSO_4$ x 7 H_20, 4.6 g $MgCl_2$ x 6 H_2O, 1.2 g $CaCl_2$ x 2 H_2O, 0.2 g $NaHCO_3$, 15 g agar (Difco), 1000 ml distilled water) containing 0.002 % cycloheximide. Colonies from the highest dilution were picked and restreaked several times for purification.

Culture conditions. Strain HEL-45^T was initially isolated from the so-called marine Cytophaga medium (DSMZ medium 172). Routine culturing using a modified Luria-Bertani agar medium, containing additional sea salts (designated LBSS; tryptone 10.0 g, yeast extract 5.0 g, NaCl 10.0 g, sea salts 14.0 g, agar 15 g, in 1000 ml of distilled water) was found to be more suitable. Working stocks of the isolate were preserved in glycerol. Storage was carried out by inoculating 5 ml of LBSS broth with a loopful of cell material and shaken for two to three days at 30 °. Aliquots of 1.5 ml of the suspension were centrifuged (7000 xg, 5 min), the supernatant was discarded. After resuspending the pellet in 500 µl of fresh LBSS broth, 750 µl of sterile glycerol (99.5 % w/v) was added and well mixed. The suspension was then equilibrated on ice for 30 minutes, followed by freezing at -18 °C for 2 hours and final storage at -70 °C. For reactivation, 50 µl of the suspension were re-cultivated by streaking on LBSS agar.

Determination of physiological characteristics. A loopfull of cell material of strain HEL-45^T was taken from a fresh culture on solid LBSS medium. A suspension corresponding to McFarland standard 1 (OD_{550} = 0.25; bioMérieux SA) was prepared in 10 ml

of saline buffer supplemented with 2% (w/v) sea salts (Sigma, Deisenhofen, Germany). The optical density was adjusted by addition of buffer or cell material respectively. One drop of this suspension was added to each of the test tubes or test plates, respectively. Incubation of the tests was performed at 25 °C.

Temperature range for growth was tested in LBSS broth from 4 °C to 60°C, halotolerance was tested in medium devoid of NaCl with 0, 1, 3, 5, 7, 10, 13, 15 % (w/v) of sea salts added. The pH range for growth was determined in a range from 5.0 to 11.0 in steps of one pH unit. The pH value was adjusted by addition of HCl or NaOH respectively. All of these tests were set up in duplicate. Capability of anaerobic growth was also tested on agar plates containing 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% gelatine were incubated with strain HEL-45^T for seven and fourteen days. Plates were then flooded with warm (55 °C) 1 N sulphuric acid, saturated with Na_2SO_4. A resulting clear circular zone around the colony indicated digestion of the gelatin. Hydrolysis of easculin was tested according to Colwell & Grigorova (1989) in a medium consisting of 10.0 g Bacto-peptone, 1.0 g Na-citrate, 1.0 g easculin and 0.05 g Fe-citrate in 1000 ml of water at a pH of 6.8 to 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 two weeks until no further growth in the test tubes was observed. Carbon sources thus tested were: glucose, acetate, propionate, butyrate, pyruvate, DL-lactate, L-aspartate, asparagine, L-glutamate, L-proline, L-serine, DL-alanine, L(+)-ornithine, succinate, methanol. Physiological reactions were also tested using the substrate panel of the API 20 NE and the API 50 CH system (Bio Mérieux SA). Additional carbon sources covered by these systems were: D-arabinose, mannose,

mannitol, N-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, phenyl-acetate, glycerol, erythritol, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methyl-xyloside, galactose, D-fructose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, amygdaline, arbutine, esculin, salicine, cellobiose, lactose, melibiose, sucrose, trehalose, inuline, melezitose, D-raffinose, amidon, glycogene, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-gluconate, and 5- keto-gluconate.

Carbon sources which tested negatively but are not included in Table 1 due to lack of data for the phylogenetic relatives are: D-arabinose, maltose, caprate, phenyl-acetate, erythritol, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methyl-xyloside, L-sorbose, dulcitol, inositol, tryptophane, α-methyl-D-mannoside, α-methyl-D-glucoside, amygdaline, arbutine, salicine, melibiose, trehalose, inuline, melezitose, D-raffinose, amidon, glycogene, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-arabitol, 2-keto-gluconate, and 5-keto-gluconate.

Reduction of nitrate, fermentation of D-glucose, presence of arginine dihydrolase, presence of urease, easculin hydrolysis, gelatin hydrolysis, and presence of β-galactosidase 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 was determined by electron microscopy. Cell morphology was investigated using 2% (w/v) agar (dissolved in water) covered slides. Transmission electron microscopic investigations were carried out as described previously (Rheims *et al*., 1999). Staining for poly-β-hydroxybutyrate was done in the following way: A heat-fixed film was prepared on a microcospic slide from a drop of culture fluid. The slide was immersed in Sudan Black B (0.3 % wt/vol in ethanol) for 5 – 15 min, drained, and air dried on blotting paper. The slide was then immersed and withdrawn in xylene several times, and blotted dry. For counterstaining, the slide was immersed in an aqueous safranin solution (0.5 % wt/vol) for 5 – 10 sec, rinsed with tap water and blotted dry. Examination was done under the light microscope with and without phase contrast. Poly-β-

hydroxybutyrate inclusion bodies appear blue-black, and the cytoplasmic parts of the organism appear pink (after counterstaining).

Chemotaxonomy. Analysis of fatty acid methyl esters was performed with 20 mg freeze-dried biomass as previously described (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 mol % G+C values by HPLC (Mesbah *et al.*, 1989) followed described procedures.

DNA-DNA hybridisation. 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 inferences. Genomic DNA was extracted from bacterial cells and purified as 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 rRNA gene sequences by PCR was done as described earlier (Pukall *et al*., 1999). Analysis of the 16S rRNA gene sequence obtained from isolate HEL-45^T 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 manually aligned and compared to sequences published previously. These were stored in the DSMZ-internal database consisting of more than 6000 16S rRNA gene 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

Colony and cell morphology. Colonies of strain HEL-45^T on agar media were whitish with a shiny surface. Single cells were irregular rods with a length of 3 to 5 μm and a width of 1.8 to 2.5 μm (Fig. 2). A few of the individual cells under microscopic investigation had a bulbus like end. Some cells were observed in stages resembling branching or budding. Strain HEL-45^T could be shown to stain Gram-negatively by classical staining technique as well as by cell lysis after the addition of 3% (w/v) KOH. As judged by microscopic investigations, HEL-45^T does not form spores. White inclusion bodies were often present, which were clearly not gas vesicles, as judged by transmission electronic investigations (Fig. 2b and further data - not shown). These inclusion bodies stained black with Sudan black, suggesting they probably consist of poly-β-hydroxybutyrate. Active motility could not be observed.

Physiological characteristics. Strain HEL-45^T grew strictly aerobically. Tests for the presence of cytochrome oxidase were repeatedly performed with fresh reagent and cultures of different age. In all cases HEL-45^T showed a weakly positive reaction. The strain did not reduce nitrate to nitrite.

Growth was poor at 8 °C, ranging to 30 °C with an optimum temperature of 25 °C to 30 °C. The pH range tolerated for growth was 7.0 to 9.0 with an optimum at pH 7.0. Strain HEL-45^T showed no growth in media devoid of salts. When only sodium chloride was added to the test media, strain HEL-45^T also failed to grow. Therefore determination of halotolerance was carried out with the addition of commercially available sea salts. Growth started at a concentration of 1 % salts, ranging to 10 % with an optimum around 3 to 5 % (w/v).

The results of further physiological tests are summarized in Table1. Also included are some of the literature data of the closest phylogenetic relatives as judged by 16S rRNA gene sequence analysis. Further carbon sources which were tested but gave negative results are listed in Methods.

Test results for hydrolysis of gelatin, starch, Tween 80, urea, and easculin were nega-

tive.

Phylogenetic inferences. Analysis of the nearly complete 16S rRNA gene sequence indicated that strain HEL-45^T shared 97.4 % sequence similarity with the sequence of *Sulfitobacter mediterraneus* DSM 12244^T. Similarity values determined for the other species of the genus *Sulfitobacte*r, as well as with *Staleya guttiformis* and *Roseobacter litoralis* ranged between 97.0 and 96.4 % (Fig. 3).

When the almost complete sequence of strain HEL-45^T was compared to those of *Sulfitobacter mediterraneus* DSM 12244^T, *Sulfitobacter pontiacus* DSM 10014^T, *Sulfitobacter brevis* DSM 11443^T, and *Staleya guttiformis* DSM 11458^T, several nucleotide positions could been identified, which can be considered as signature nucleotides comprising specific base-pair exchanges. At the four positions 132-230 (G:C); 140-223 (C:G); 144-178 (G:C); 1356-1366 (G:C), given according to the *E. coli* nomenclature (Brosius *et al*., 1978), base pairs of HEL-45^T were different from those of the other *Sulfitobacter* species or *Staleya guttiformis*. For those strains the following combinations at the same positions were found: 132-230: (A:U, all); 140-223 (*S. mediterraneus* U:G, others: U:A); 144-178 (A:U, all); 1356-1366 (U:A, all).

DNA:DNA relatedness determined by the spectrophotometric method revealed only low similarity value of 21.2 % between strains HEL-45^T and *Sulfitobacter mediterraneus* DSM 12244^T. The G+C content of the DNA was determined to be 60.1 mol % for strain HEL-45^T.

Chemotaxonomic properties. Analysis of the respiratory quinone composition of strain HEL-45^T, *Sulfitobacter pontiacus* DSM 10014^T, *Sulfitobacter brevis* DSM 11443^T, *Sulfitobacter mediterraneus* DSM 12244^T, and *Staleya guttiformis* DSM 11458^T indicated that in all strains Q10 predominated. The presence of Q10 as the dominant respiratory quinone is a feature of many, but not all members of the Alphaproteobacteria. Although Q10 is also found in *Legionella* species (Gammaproteobacteria subclass) it is not the sole, major component in these bacteria, making the presence of Q-10 as the sole major respiratory quinone specific for members of the Alphaproteobacteria.

The polar lipid composition of all strains showed a high degree of similarity, with phospholipids comprising phosphatidyl glycerol, phosphatidyl choline and phosphatidyl ethanolamine be present. In addition an aminolipid was also present. Diphosphatidyl glycerol was not universally present. The fatty acid composition of all strains gave patterns in which 18:1ω7c predominated, but different groupings could be distinguished on the basis of the remaining fatty acids which are listed in Table 2.

As discussed earlier by Labrenz et al. (2000) we have been able to confirm some degree of infra structure within this group based on the polar lipid and fatty acid composition. We observed some differences in the relative percentages in the fatty acid composition compared to previous work, although small changes are to be expected in the relative composition due to slight differences in conditions of cultivation and should not be over emphasised when evaluating the data. Closer examination of the fatty acid composition revealed three major groups: (1) *Sulfitobacter pontiacus* DSM 10014^T/*Sulfitobacter brevis* DSM 11443^T, (2) strain HEL-45^T, and (3) *Staleya guttiformis* DSM 11458^T/*Sulfitobacter mediteraneus* DSM 12244^T. In particular, although all members of this group produced 3-OH 10:0 there were differences in the distribution of other 3-OH fatty acids. It should be noted that unambiguous proof is not presented here that the 3-OH 12:1 or 3-OH 14:1 are not 3-oxo derivatives which are very difficult to distinguish on the basis of mass spectrometry alone. *Sulfitobacter pontiacus* DSM 10014^T and *Sulfitobacter brevis* DSM 11443^T produced a 3-OH 12:0 and 3-OH 14:1 (or 3-oxo 14:0), whereas *Staleya guttiformis* DSM 11458^T and *Sulfitobacter mediteraneus* DSM 12244^T produced only the 3-OH 14:1 (or 3-oxo 14:0), and strain HEL-45^T produced 3-OH 12:1 (or 3-oxo 12:0). In the case of the fatty acids identified here as 3-OH 12:1 (or 3-oxo 12:0), 3-OH 12:0, and 3-OH 14:1 (3-oxo 14:0) they all appeared to be amide linked.

Although it may not be possible to determine with absolute certainty the branching order within the group defined by members of the genera *Staleya-Sulfitobacter*- strain HEL 45^T based on the 16S rRNA gene sequences, 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). It is particularly interest-

ing that *Sulfitobacter pontiacus* DSM 10014^T/*Sulfitobacter brevis* DSM 11443^T, strain HEL-45, and *Staleya guttiformis* DSM 11458^T/*Sulfitobacter mediteraneus* DSM 12244^T form three distinct groups based on the chemical composition.

The data presented here are consistent with what has been reported before in the literature, where the absolute branching order of taxa showing short internal branches cannot be determined unambiguously based on the 16S rRNA gene sequence data alone. Ludwig et al. (1998) have proposed that in such cases the branches should be collapsed to give a "collapsed clade." In such cases the 16S rRNA gene sequence data can only give a polychotomy at best, which would suggest that this group of strains represents a single genus. However, the chemical composition clearly indicates that there is infrastructure within this group and may be used to define at least three subgroupings. These groupings appear to be no less significant than those defined by other genera where the chemical composition, 16S rRNA gene sequences and biochemical/physiological data have been taken into consideration in this subgroup of the Alphaproteobacteria (see Labrenz et al., 1998; 1999; 2000). It should be noted that the 16S rRNA gene sequence similarity between the strains is greater than 96%, suggesting that the often used value of 95% similarity for delineating genera would not take into account the chemical diversity of this group. In fact, using the value of 95% similarity as a cut-off value would imply that all these species should be placed in the genus *Roseobacter*.

In the case of strain HEL-45^T we interpret the chemical composition of the cell as being indicative of the fact that this strain, which obviously represents a novel species, should also be placed in a new genus. The chemical heterogeneity evident within the genus *Sulfitobacter*, as currently defined, would justify transferring *Sulfitobacter mediterraneus* to the genus *Staleya*. However, the ability for aeobic anoxygenic photosynthesis has been found in *Staleya* and is a significant physiological trait that has to be weighed against the chemical composition of the cells (Yurkov & Beatty, 1998). In the absence of additional information, and in view of the needs of the end users of bacterial systematics, we therefore refrain at present from transferring *Sulfitobacter mediterraneus* to the

genus *Staleya*.

Description of *Oceanibulbus* gen. nov.

O.ce.a`nus N.L. m. the sea; bul`bus N.L. m. onion, referring to the occasionally swollen ends of individual cells; *Oceanibulbus* onion like bacterium from the sea.

Gram negative, non motile irregular rods with a tendency to form slightly swollen ends. On LBSS agar, the strain develops colonies within 3 to 5 days. They do not form spores. Bacteriochlorophyll *a* is not produced. Growth is poor at 15°C and optimal at 25 – 30°C. The pH optimum for growth is 7.0 – 8.0. Strictly aerobic, non fermentative heterotrophs. Inclusion bodies are often present. In media devoid of salts or containing only sodium chloride no growth is observed. Growth starts at 1 % sea salts up to 7 % (w/v). They show a weakly positive reaction in tests for cytochrome oxidase, and do not reduce nitrate to nitrite.

The predominant respiratory quinone present is ubiquinone 10. The major polar lipds are phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidyl choline, phosphatidyl ethanolamine, and an aminolipd. The fatty acids comprise 18:1ω7c, 18:0, 16:1ω7c, 16:0, 3-OH 10:0, 3-OH12:1 (or 3-oxo 12:0), and traces of an 18:2 fatty acid. Among the hydroxylated fatty acids only the 3-OH 12:1 (or 3-oxo 12:0) appears to be amide linked, whereas the 3-OH 10:0 appears to be ester linked.

The strains were originally obtained from a North Sea water sample from a depth of 10 m. The type species for the genus is *Oceanibulbus indolifex.*

Description of *Oceanobulbus indolifex* sp. nov.

Oceanibulbus indolifex (in.do`li.fex N.L. masc. n. *indolum*, indole; N.L. masc. suff. -*fex*, from L.v*. facio*, to make; M.L. adj. *indolifex* making indole/ the indole maker).

Gram-negative, non-spore-forming, strictly aerobic and heterotrophic bacteria. Cells are non-motile irregular rods with a variable size of 3 – 5 μm length and 1.8 – 2.5 μm width. Some cells have a swollen end. They have inclusion bodies which appear white in transmission electron microscopic sections and consist of poly-β-hydroxybutyrate. They

are catalase positive, and weakly oxidase positive. The GC content is 60.1 %. Cells grown on LBSS develop small, whitish, shiny colonies within 3 to 5 days. Optimal growth occurs at 25°C – 30°C, slow growth was observed at 15°C. The pH range tolerated for growth was 7.0 – 8.0. No growth was observed in media without salts or only sodium chloride. When supplemented with commercially available sea salts, growth started at a concentration of 1 %, ranging to 7 % (w/v). Hydrolysis of gelatin, starch, Tween 80, urea, and easculin was not observed. The strains did 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 showed utilization of D-glucose, pyruvate, DL-lactate, serine, ornithine, alanine, asparagine, L-aspartate, L-glutamate, L-proline, succinate,

mannitol, adipate, malate, citrate, and glycerol. Chemotaxonomic properties and other characteristics are as for the genus. The type strain *Oceanibulbus indolifex*, strain HEL-45^T has been deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen as DSM 14862^T (www.dsmz.de) and at the NCIMB (National Collections of Industrial, Food and Marine Bacteria, www.ncimb.co.uk) as NCIMB 13983. The EMBL accession number of the 16S rRNA gene sequence is AJ550939.

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Legends to Tables and Figures

Table 1 Selected physiological properties of strain HEL-45^T compared with its closest phylogenetic relatives (as judged by 16S rRNA gene sequence analysis), *Sulfitobacter mediterraneus*, *S. pontiacus*, *S. brevis*, and *Staleya guttiformis*.

Table 2 Pecentage composition of the fatty acids present in strain HEL-45^T . Method1 : ester-linked fatty acids; Method 2: ester- and amide-inked fatty acids (see Tindall 1990 a,b). Presumptive amide linked fatty acids are labelled with *.

Figure 1 Secondary metabolites purified and identified from cultures of HEL-45^T. 1 cyclo-(phe,pro); 2 cyclo-(tyr, pro); 3 cyclo-(leu, pro); 4 tryptanthrin; 5 indole-3-carboxylic acid; 6 3-indole-carbaldehyde; 7 indole-3-carboxylic acid thiomethyl ester; 8 3,3-bis-(indol-3-yl)-propane-1,2-diol.

Figure 2 Microscopic images of cells of strain HEL-45^T. (A) Light microscopic image. The scale bar indicates 15 μm; (B) Transmission electron microscopic (TEM) image. The scale bar indicates 1.3 μm.

Figure 3 Dendrogram of 16S rRNA gene sequence relatedness showing the positions of strain HEL-45^T next to its nearest identified neighbours within the alpha-subclass of the class Proteobacteria. The tree was calculated by the neighbor-joining method from almost complete sequences. The percentage of 1000 bootstrap resamplings that support branching points above 65 % confidence is indicated. The scale bar represents 10 nucleotide substitutions per 100 sequence positions. The tree was rooted with *E. coli* as an outgroup. Sequence accession numbers are given in brackets.

Table 1

Characteristic	HEL-45[T]	*Sulfitobacter mediterraneus*[1]	*S. pontiacus*[2]	*S. brevis*[3]	*Staleya guttiformis*[3]
Cell morphology	irregular rod	rod	rod	rod	Rod
Cell size	1.8-2.5 x 3-5 μm	1-3 x 0.5-0.8 μm	0.45-1.3 x 2-5 μm	0.8-1.0 x 1.1-1.5 μm	1.0-1.5 x 1.5-8.9 μm
Motility	-	+	+	+	+
Flagella	-	1-5, subpolar	1-5, subpolar	-	+
Catalase	+	+	+	+	w
Oxidase	w	+	+	+	+
Bchl *a*	-	-	-	-	+
Mole % G+C	60.1	59	62.1	58	55.0-56.3
Carbon source utilization:					
D-glucose	+	+	ND	w	w
Acetate	-	+	+	+	+
Propionate	-	+	+	ND	ND
Butyrate	-	+	+	+	-
L-Proline	w	+	+	-[5]	-[5]
Serine	+	+	+	w[5]	-[5]
Alanine	+	+	+	-[5]	-[5]
L(+)-Ornithine	+	+	+	-[5]	-[5]
API/BIOLOG reactions:					
Arginine	-	ND	+	ND	ND
D-Mannose	-	+	ND	+	-
D-Mannitol	+	+	ND	w	-
N-Acetyl-glucosamine	-	ND	ND	+	-
Gluconate	-	+	+	ND	ND
Adipate	+	ND	ND	ND	ND
Citrate	+	+[4]	-	+	-
D-Galactose	-	ND	ND	+	-
D-Fructose	-	ND	ND	+	w
L-Rhamnose	-	ND	ND	+	w
D-Sorbitol	-	ND	ND	+	-
Cellobiose	-	ND	ND	w	-
α-Lactose	-	ND	ND	+	w
Sucrose	-	ND	ND	+	w
L-Fucose	-	ND	ND	+	w
D-Arabitol	-	ND	ND	+	-

+, positive reaction / growth detectable; w, weakly positive reaction; -, negative reaction / no growth detectable; ND, not determined.
[1], data according to Pukall *et al.* (1999).
[2], data according to Sorokin (1995).
[3], data according to Labrenz *et al.* (2000).
[4], stimulated in media containing yeast extract or biotin
[5],.determined in BIOLOG microtitre plates

Table 2

Fatty acid	HEL-45^T		*Staleya guttiformis* DSM 11458^T		*S. mediterraneus* DSM12244^T		*S. pontiacus* DSM10014^T		*S. brevis* DSM11443^T	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
3-OH 10:0	1,612	2,698	1,561	2,286	2,265	3,062	1,787	2,851	1,693	2,391
3-OH 12:1 (or 3-oxo 12:0)	-	1,209*	-	-	-	-	-	-	-	-
3-OH 12:0	-	-	-	-	-	-	-	0,657*	-	0,623*
3-OH 14:1 (or 3-oxo 14:0)	-	-	-	1,521*	-	2,08*	-	1,044*	-	0,791*
16:1ω7c	1,869	2,022	-		-		-	0,841	-	-
16:0	4,658	5,244	4,353	5,798	5,665	6,417	9,972	10,099	11,57	14,728
18:2	-	-	-	-	-	-	-	-	0,839	0,745
18:2	0,693	0,677	-	-	3,242	2,799	-	-	5,273	4,569
18:1	-	-	-	-	-	-	-	-	7,329	7,536
18:1ω7c	88,38	85,19	86,862	83,506	81,955	77,735	78,86	77,365	68,146	62,173
not identified	-	-	-	-	-	-	1,996	-	-	-
18:0	-	-	1,341	1,214	1,973	2,013	1,322	1,16	2,135	2,161
not identified	1,653	1,568	2,033	1,823	3,706	3,576	5,274	5,036	1,959	1,979
not identified	1,135	1,392	1,358	1,481	1,194	1,505	-	0,947	1,056	1,315
cyclo 19:0	-	-	2,492	2,371	-	-	-	-	-	-

Fig. 1

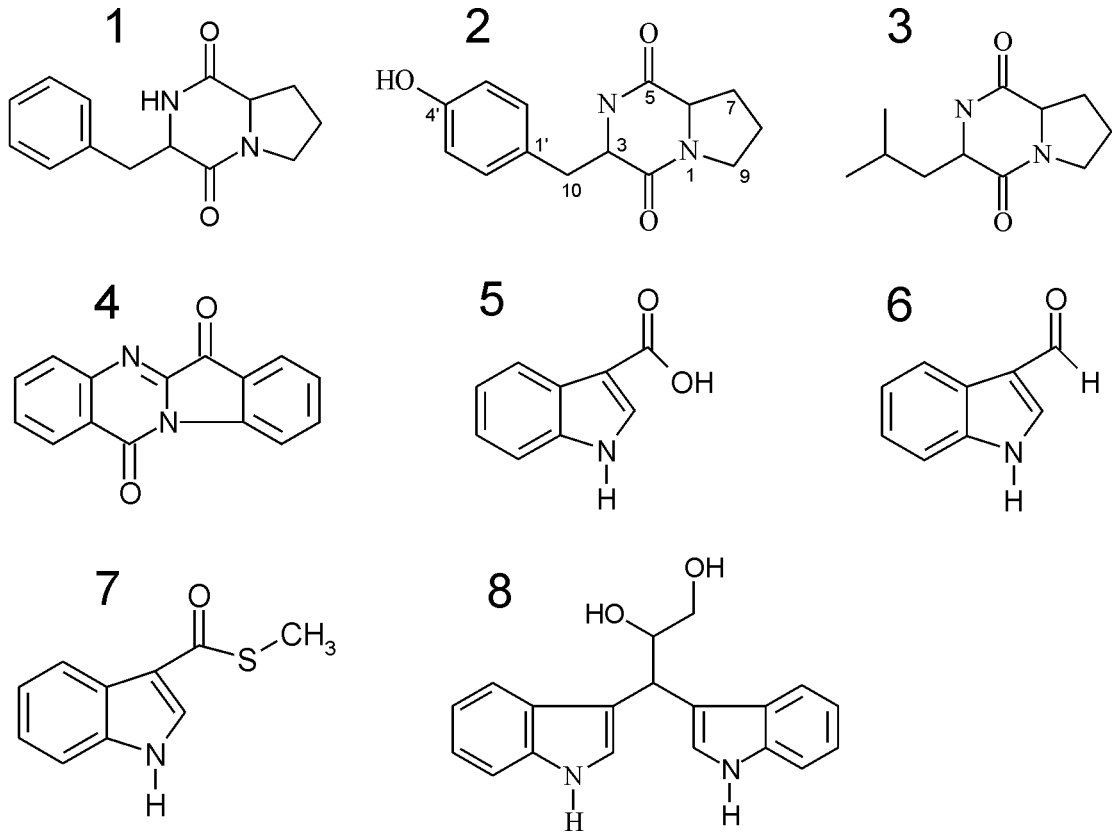
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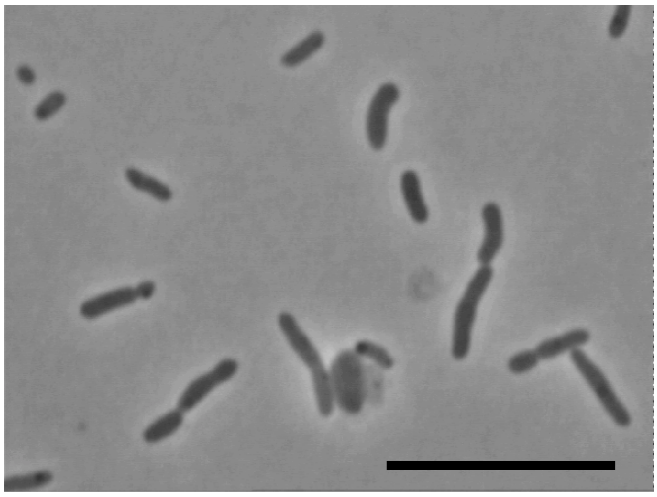
Fig. 2



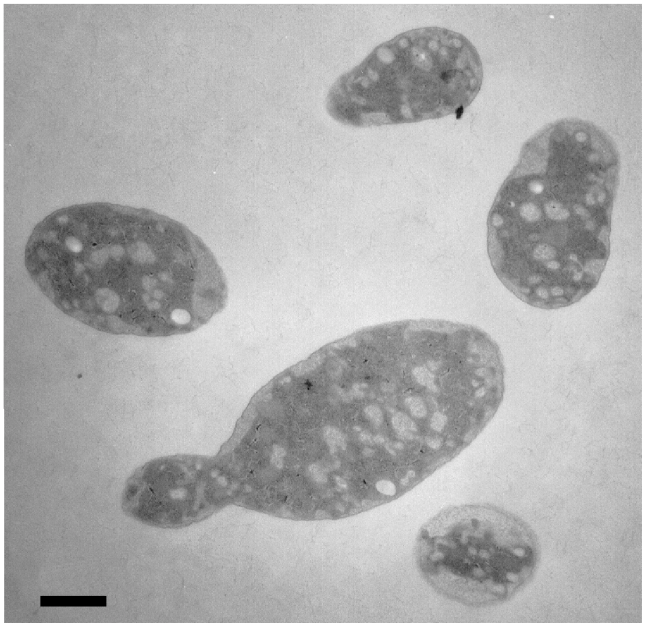
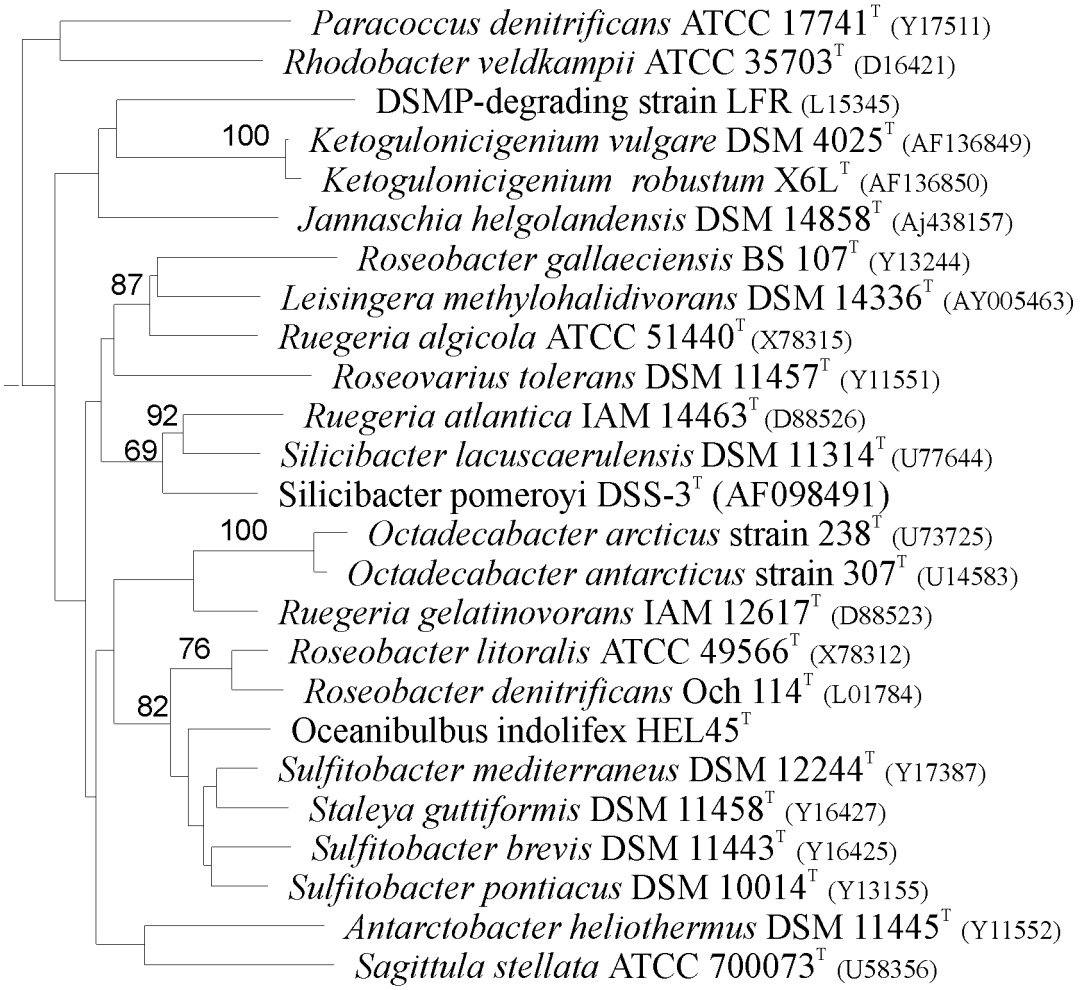


Fig. 3


Paracoccus denitrificans ATCC 17741^{T} (Y17511)
Rhodobacter veldkampii ATCC 35703^{T} (D16421)
DSMP-degrading strain LFR (L15345)
100
Ketogulonicigenium vulgare DSM 4025^{T} (AF136849)
Ketogulonicigenium robustum X6L^{T} (AF136850)
Jannaschia helgolandensis DSM 14858^{T} (Aj438157)
Roseobacter gallaeciensis BS 107^{T} (Y13244)
87
Leisingera methylohalidivorans DSM 14336^{T} (AY005463)
Ruegeria algicola ATCC 51440^{T} (X78315)
Roseovarius tolerans DSM 11457^{T} (Y11551)
92
Ruegeria atlantica IAM 14463^{T} (D88526)
69
Silicibacter lacuscaerulensis DSM 11314^{T} (U77644)
Silicibacter pomeroyi DSS-3^{T} (AF098491)
100
Octadecabacter arcticus strain 238^{T} (U73725)
Octadecabacter antarcticus strain 307^{T} (U14583)
Ruegeria gelatinovorans IAM 12617^{T} (D88523)
76
Roseobacter litoralis ATCC 49566^{T} (X78312)
Roseobacter denitrificans Och 114^{T} (L01784)
82
Oceanibulbus indolifex HEL45^{T}
Sulfitobacter mediterraneus DSM 12244^{T} (Y17387)
Staleya guttiformis DSM 11458^{T} (Y16427)
Sulfitobacter brevis DSM 11443^{T} (Y16425)
Sulfitobacter pontiacus DSM 10014^{T} (Y13155)
Antarctobacter heliothermus DSM 11445^{T} (Y11552)
Sagittula stellata ATCC 700073^{T} (U58356)
.10