Tritonibacter horizontis gen. nov., sp. nov., a member of the *Rhodobacteraceae*, isolated from the Deepwater Horizon oil spill

Franziska Klotz,[1]† Thorsten Brinkhoff,[1] Heike M. Freese,[2] Matthias Wietz,[1] Andreas Teske,[3] Meinhard Simon[1] and Helge-Ansgar Giebel[1,*]

Abstract

A heterotrophic, Gram-stain-negative, aerobic, sodium-requiring and motile bacterium was isolated from oil-contaminated surface water of the Gulf of Mexico during the Deepwater Horizon oil spill. Strain O3.65^T showed highest 16S rRNA gene sequence similarity to *Phaeobacter gallaeciensis* BS107^T and *Phaeobacter inhibens* T5^T, both with 98.3 %, respectively. Based on complete genome analysis, highest similarity was observed to species of the genus *Ruegeria*. Strain O3.65^T exhibited a broad salinity, temperature and pH range of 0.5–10 % NaCl, 4–45 °C and 5.5–9.0, respectively. The DNA G+C content of strain O3.65^T was 61.5 mol%. The major respiratory lipoquinone was ubiquinone-10 (Q-10), the most dominant fatty acids (>1 %) comprised 18 : 1ω7*c* and 18 : 1ω7*c* 11-methyl, 10 : 0 3OH, 12 : 1 3OH, 14 : 1 3OH/3-oxo-14 : 0, 16 : 0, 16 : 0 2OH, 18 : 1 2OH and 12 : 1. The polar lipid pattern indicated presence of phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid, two unidentified phospholipids and seven unidentified lipids. On Difco marine broth agar, strain O3.65^T formed smooth, shiny white to beige and convex colonies with regular edges. Phylogenetic, phylogenomic and phenotypic differences revealed that strain O3.65^T represents a new species of a novel genus within the family *Rhodobacteraceae*, for which we propose the name *Tritonibacter horizontis* gen. nov., sp. nov. The type strain of the type species is O3.65^T (=DSM 101689^T=LMG 29740^T).

The largest oil spill in US history, the Deepwater Horizon (DWH) oil rig explosion in the Gulf of Mexico (GOM) had a major impact on the bacterial community by reducing the diversity and resulting in a temporal succession of different bacterial phyla in the course of the oil degradation process [1–4]. For possible human bioremediation interventions in future spills, it is essential to know which bacteria are involved in the actual process of degrading oil. A so far neglected group of bacteria in relation to oil spills is the family *Rhodobacteraceae* within the *Alphaproteobacteria*. Most species of the globally distributed and abundant *Rhodobacteraceae* are affiliated with the ecologically important marine '*Roseobacter* group' [5], are heterotrophs being able to use various organic carbon sources by their high diversity of metabolic capabilities [6–8]. Due to their physiological versatility, they occupy various habitats and ecological niches. Culture-independent studies revealed that members of the *Rhodobacteraceae* increase in numbers after enrichment with a range of hydrocarbons, and showed that genes encoding for enzymes involved in aromatic and aliphatic hydrocarbon degradation are prevalent in their genomes [9]. However, compared to other sources, isolations of members of the family *Rhodobacteraceae* from oil-contaminated environments are rarely reported [10–14]. To our knowledge, there are no *Rhodobacteraceae* species with valid names that have been obtained from oil-contaminated samples of the DWH accident. Strain O3.65^T was isolated from oil-contaminated enrichment cultures of DWH surface water. Interestingly, a clone obtained by Arnosti *et al.* [4] from oily aggregates grown in an incubation experiment amended with DWH oil and an undescribed strain from a biotrap in DWH contaminated waters showed a 16S rRNA gene sequence similarity of 99 % and even 100 %, respectively, to strain O3.65^T, pointing to its possible contribution in the oil degradation network during the spill. Furthermore, strain O3.65^T was able to grow on several hydroxylated and substituted aromatic compounds, but was lacking genes coding for enzymatic degradation of alkanes [15]. Phylogenetic and additional phylogenomic analysis revealed that strain O3.65^T cannot be

Author affiliations: [1]Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, D-26111 Oldenburg, Germany; [2]Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; [3]Department of Marine Sciences, University of North Carolina, Chapel Hill, NC, USA.
***Correspondence:** Helge-Ansgar Giebel, giebel@icbm.de
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Abbreviations: dDDH, digital DNA–DNA hybridization; DMSO, dimethyl sulfoxide; DMSP, dimethylsulfoniopropionate; DWH, Deepwater Horizon; GOM, Gulf of Mexico; MB, marine broth; MM, mineral medium; TDA, tropodithietic acid.
†Present address: Department of Biology, University of Konstanz, 78464 Konstanz, Germany.
The GenBank accession number for the genome sequence of *Tritonibacter horizontis* O3.65^T is LPUY00000000.1. The locus_tag including the 16S rRNA gene sequence is TRIHO_RS00090.
One supplementary figure and two supplementary tables are available with the online version of this article.

classified as a member of a known genus of the family *Rhodobacteraceae* [15].

Here we describe the new isolate, strain O3.65^T, obtained from surface water of the DWH oil spill. Based on a comprehensive characterization, strain O3.65^T is proposed to be a representative of a new species of a new genus within the family *Rhodobacteraceae*.

Strain O3.65^T was obtained from surface water contaminated with crude oil close to the Macondo wellhead in the Gulf of Mexico (28° 43′ 58.0″ N, 88° 22′ 59.6″ W), collected on June 1st, 2010 during the oil spill. Further details of the sampling site and the isolation were given before by Giebel *et al.* [15]. Cultivation of strain O3.65^T was done with marine broth medium (MB; Difco 2216) or mineral medium (MM) after Zech *et al.* [16], supplemented with 1 ml l^{-1} trace element solution and 1 ml l^{-1} multivitamin solution after Martens *et al.* [17]. If not stated otherwise, all tests were done in triplicates at 20 °C in MM with glucose as sole carbon source with a final concentration of 5 mM in non-shaken test tubes in the dark. Strain O3.65^T was stored in liquid MB supplemented with 30 %-glycerol at −80 °C.

For bacteriochlorophyll *a* determination, cells were grown at 15 °C in a light–dark rhythm until late exponential phase and extracts prepared after Ruivo *et al.* [18]. Absorption and transmission was measured at a spectrum of 350–800 nm with a spectrophotometer (PhotoLab 6600 UV-VIS series; Fisher Scientific). *Dinoroseobacter shibae* DFL-12^T served as a positive control. Genomic analysis of strain O3.65^T revealed absence of a photosynthetic operon for the ability of aerobic anoxygenic photosynthesis [15], but was checked additionally by a specific PCR described elsewhere [19].

Growth response regarding temperature was determined from 4–45 °C (from 10 °C on in 5 °C steps) in test tubes with 5 ml MM. Growth was monitored by measuring the optical density at a wavelength of 600 nm (OD_{600}) as a proxy for growth. The response on different pH values was tested in the range of 4.0–10.5 in increments of 0.5; adjusted with sterile NaOH or HCl after Hahnke *et al.* [20]. A salinity range from 0 to 10 % (0, 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.5, 8 and 10 % w/v) was prepared after Hahnke *et al.* [20] and tested for growth. Na^+ requirement was determined by preparing medium without a Na^+ source, as follows (l^{-1}): 20.5 g KCl, 4.0 g $MgSO_4 \times H_2O$, 3.0 g $MgCl \times H_2O$, 0.39 g KH_2PO_4, 0.25 g NH_4Cl and 0.15 g $CaCl \times 2H_2O$ were dissolved in distilled water. The medium was supplemented with vitamins and trace elements, as stated above.

Requirement for the vitamins biotin, cobalamin, nicotinic acid amide, pantothenic acid, pyridoxal hydrochloride, riboflavin and thiamine (all at 0.05 mg l^{-1}) was tested in MM, whereas in different assays one vitamin was omitted. Cells were washed two times in MM without any vitamins. Cells were transferred twice into fresh media to verify the ability to grow without the specific vitamins.

Substrate utilization of strain O3.65^T was tested with 50 carbon sources in 5 ml MM supplemented with the respective substrate. All tested substrates are listed in the species description. The final concentration was 1 g l^{-1} for carbohydrates, 1 mM for amino acids, 100 µM for DMSP and DMSO, 1 % (v/v) for alkanes and 0.01 % (w/v) for aromatic acids, except for benzoic acid, 4-hydroxy-benzoic acid, 3,4-dihydroxy-benzoic acid, it was 0.005 % (w/v). The other carbon sources were tested with a final concentration of 1 mM. Cells were transferred twice into fresh medium to verify the ability to grow on the specific substrate. Furthermore, strain O3.65^T was tested for pure crude oil degradation on plates containing 0.2 % oil from the *in situ* samples of its isolation as well as for paraffin (1 %).

Antibiotic susceptibility to ampicillin, chloramphenicol, gentamicin, kanamycin, penicillin G and streptomycin was analysed at concentrations of 20 and 100 µM and in addition 1 mM for ampicillin and kanamycin in MM.

Growth rate (µ) and doubling time (t_d=ln2/µ) were determined for strain O3.65^T under the following conditions: 20 °C, pH 7.6, salinity 31 psu, at 104 r.p.m. in baffled Erlenmeyer flasks filled with 100 ml MB in the dark. Media were inoculated with 1 % (v/v) of a pre-culture growing in exponential phase. Growth was monitored by measuring OD_{600} every 1–2 h. Growth rate and doubling time were determined by linear regression of semi-logarithmic plots of optical density against time.

The Gram-staining was conducted after Gregersen [21]. Cytochrome catalase and oxidase activities were tested as described by Smibert and Krieg [22] with *Pseudomonas fluorescens* (DSM 50090^T) as positive control. Exoenzyme activities, i.e. hydrolysis of gelatin, starch and Tween 80 were analysed after Smibert and Krieg [22] on MM plates, solidified with either 9 % (w/v) gelatin or 1.5 % (w/v) agarose supplemented with 0.2 % (w/v) starch and 1 % (v/v) Tween80, respectively, at 20 °C and additionally for gelatin degradation at 15 °C. Anaerobic growth with nitrite was tested in anoxic mineral base medium after Cypionka and Pfennig [23] with 0.5 g resazurine (0.5 mg ml^{-1}) supplemented after autoclaving with trace element solution SL10 (1 ml, [24]), vitamin solution V10 (1 ml, [25]), selenite and tungstate solution (0.1 mM; 0.2 ml, [26]) $NaHCO_3$ (30 ml) and 5 mM glucose as carbon source. Cultures were supplemented with 5 mM nitrite as electron acceptor and left in the dark at 20 °C for nine weeks. Reduction of nitrite was determined at weeks 4 and 9 photometrically after Griess [27]. *Leisingera nanhaiensis* DSM 24252^T served as a positive control.

Colony morphology and possible motility were checked by light microscopy of cells after 5 days of incubation at 20 °C grown in MM medium (Zeiss Axio Lab1). For transmission electron microscopy (TEM) cells were taken from the exponential phase, growing on 5 mM glucose using prefiltered (0.22 µm) medium and processed as described elsewhere [19].

The 16S rRNA gene sequence of strain O3.65^T was obtained after Giebel *et al.* [19]. Phylogenetic 16S rRNA gene tree construction was done with the ARB software (www.arb-home.de; [28]) taking into account all closely related type strains with at least 97 % sequence similarity as indicated by BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as well as the type species of the related genera. Digital DNA–DNA hybridization (dDDH) between strain O3.65^T and genomes of related type strains was calculated by the Genome-to-Genome Distance Calculator with formula 2 [29, 30]. The phylogenomic tree is based on amino acid sequences of 1475 core genes identified using BPGA [31], which were aligned with MUSCLE [32]. The best substitution model (LG+I+G+F) was computed using ProTest 3 [33]. A maximum-likelihood tree with 1000 bootstrap replicates was calculated using RaxML [34] implemented on the CIPRES Science Gateway [35].

Chemotaxonomic analyses were done as described previously [36–38]. Fatty acid methyl esters were released from 20 mg freeze dried cells from the late exponential phase grown in MB medium according to the Microbial Identification System (MIS) standard protocol [39]. Compounds were identified by comparison against the TSBA40 peak naming table database. These analyses were carried out in the same laboratory that also characterized most of the currently available and here relevant reference type strains, which were grown all in MB medium (i.e. *Phaeobacter inhibens* T5^T, *Phaeobacter gallaeciensis* BS107^T, *Leisingera methylohalidivorans* MB2^T, *Ruegeria atlantica* 1480^T; [17]).

Using an inoculum of oil-polluted DWH seawater that had been stored for 4 years at 4 °C, strain O3.65^T was isolated using MB agar plates. Single cells of strain O3.65^T are ovoid rods of 1.3–2.2 µm length and 0.6–1.0 µm width and Gram-stain-negative. Light microscopic analysis revealed that single cells were motile, whereas chains and aggregates seemed to be non-motile. Motility is accomplished with a bundle of polar flagella, which were visible in transmission electron microscopy (Fig. 1). In minimal medium strain O3.65^T formed rosette-shaped aggregates in late exponential and stationary phase, which are typical for members of the *Roseobacter* group [6] and facilitate to colonize surfaces and build biofilms [40, 41]. On MB medium colonies are smooth, convex with regular edges and of white to beige colour. No spore formation was observed. Cells were not pigmented; bacteriochlorophyll-*a* and genes coding for subunits of the photosynthetic reaction centre were not found in strain O3.65^T.

During exponential growth phase strain O3.65^T grew with a rate of ~0.13 h^{-1} and exhibited a doubling time of 5.2 ±0.2 h^{-1}. No growth was observed when Na^+ was absent, showing requirement for sodium and indicating a marine mode of life. Salinity, temperature and pH-ranges and optima, respectively, are listed in the species description. Strain O3.65^T required the vitamin biotin for growth, which was underscored by genomic analysis [15]. Strain O3.65^T grew on various carbon sources, including hydroxylated aromatic acids. All tested substrates are listed in the species description.

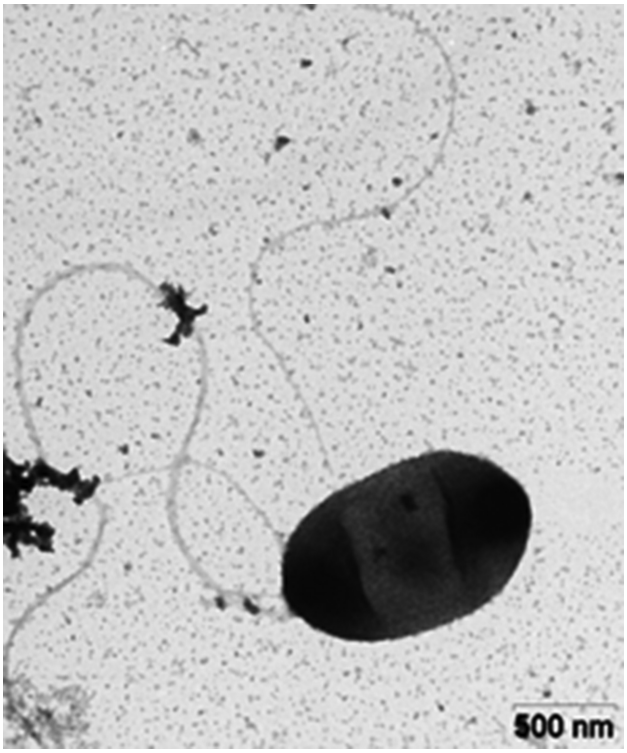

Fig. 1. Transmission electron micrograph showing a single cell of strain O3.65^T. Note the long bundle of polar flagella. Bar 500 nm.

No resistance was detectable against any of the antibiotics at any concentration tested, with the exception of kanamycin. Here, growth of strain O3.65^T was detectable at a concentration of 20 µM, but not at the higher concentrations of 0.1 and 1.0 mM. The essential gene coding for the enzyme for kanamycin resistance, neomycin phosphotransferase II (*npt*II; EC 2.7.1.95) was not present in the genome of strain O3.65^T. Possibly, strain O3.65^T was not resistant against kanamycin, but could tolerate low concentrations ($\leq$20 µM).

Phylogenetic analyses based on 16S rRNA gene sequences of related type strains demonstrated that strain O3.65^T forms together with two full-length sequences (>1300 bp) of one environmental clone and one uncharacterized strain a distinct monophyletic cluster, well separated from representatives of the genera *Phaeobacter*, *Pseudophaeobacter*, *Leisingera* and *Ruegeria* (Fig. 2). The branch-off is supported by a moderate bootstrap value (62 %) and supported by maximum likelihood calculation. Strain O3.65^T clusters together with the undescribed strain *Ruegeria* species 39RL_GOM-46m (SRX711597) obtained from an oil-amended biotrap and clone Oil-BE-016 from an oil slick sample of a laboratory incubation experiment [4, 15]. Both are gained from the DWH oil spill and have a high sequence similarity of 100 and 99 %, respectively. The closest type strains are *P. gallaeciensis* BS107^T and *P. inhibens* T5^T, both

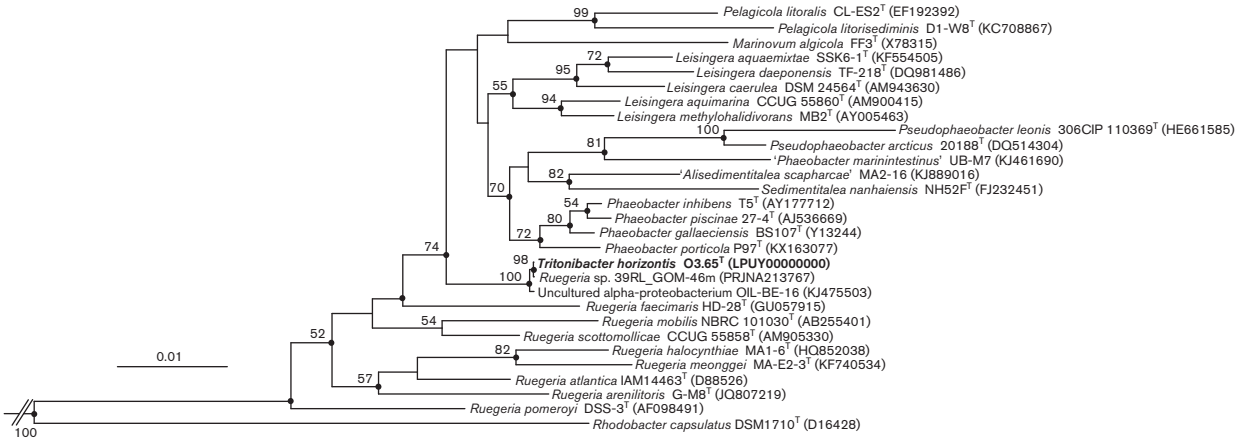

Fig. 2. Neighbour-joining tree highlighting the phylogenetic position of strain O3.65^T relative to closely related type strains of up to 97 % 16S rRNA gene sequence similarity and the respective type species of related genera within the *Rhodobacteraceae*. The tree was calculated with nearly full-length 16S rRNA gene sequences (≥1350 bp). Only bootstrap values ≥50 % (derived from 1000 replicates) are shown. Filled circles indicate nodes also recovered reproducibly with maximum-likelihood (PHYML) calculation. *Synechococcus* strains (AY946243, CP000951, AF448073, not shown) served as an outgroup. Bar, 0.01 substitutions per nucleotide position.

with 98.3 % 16S rRNA gene sequence similarity, followed by *Ruegeria scottomollicae* CCUG55858^T (98.1 %) and *Leisingera aquimarina* CCUG 55860^T (98.0 %). For all type species of the related genera, e.g. *Leisingera, Ruegeria* and *Pseudophaeobacter*, the sequence difference is even higher (≥2.0 %; Table S1, available in the online version of this article). This diverse phylogenetic mixture of the next closest related strains reflects the insufficient resolution of the 16S rRNA gene for accurate classification of strain O3.65^T. However, that issue is frequently found between and within subclusters of the *Rhodobacteraceae*, which have low divergence of the 16S rRNA gene accompanied by high genomic divergence [42]. In this case the high genomic divergence is reflected by a distinct very low mean similarity (20.4±2.2 %) by dDDH analysis for strain O3.65^T compared to the available genomes of 17 related type strains and is based on updated previously published data [15] shown in Table S1. Using the 70 % criterion [43] this indicates that strain O3.65^T is not a member of the most closely related species tested among the genera *Phaeobacter*, *Pseudophaeobacter*, *Leisingera* and *Ruegeria*. Thus, the genomic repertoire of strain O3.65^T differs significantly compared to its closely related neighbours including type strains. High similarity based on dDDH was found only for the genome of strain *Ruegeria* species 39RL_GOM-46m with a maximal value of 100±0.1 %, implying that strain 39RL_GOM-46m is another strain of the newly proposed species *Tritonibacter horizontis* but does not belong to the genus *Ruegeria*.

Furthermore, a previously published comprehensive genome analysis revealed that strain O3.65^T is lacking one of the most prominent *Phaeobacter*-specific characteristics, i.e. the production of tropodithietic acid (TDA) and brownish pigmentation [15, 44], what supports the separation from the genus *Phaeobacter*. Comparative phylogenomic analysis of 1475 core genes of the genomes of strain O3.65^T, of strain 39RL_GOM-46m and the additional genomic data of 17 type strains, affiliated with the genera *Phaeobacter*, *Pseudophaeobacter*, *Leisingera, Ruegeria* and *Roseobacter*, showed a clear separation of strain O3.65^T together with strain 39RL_GOM-46m to the clusters of the above-mentioned genera (Fig. 3), supporting the phylogenetic analysis based on 16S rRNA gene sequences (Fig. 2). Strain O3.65^T and strain 39RL_GOM-46m share 807 unique genes. As shown by a previously published study [15], the phylogenetic location of strain O3.65^T is also supported by highest bootstrap values of 100 %. Interestingly, here also a single *Ruegeria* strain, *Ruegeria scottomollicae* CCUG55858^T, was separated from other *Ruegeria* type strains and branched adjacent to the *Tritonibacter* cluster. But dDDH analysis revealed only ~22.2 % similarity on genomic level between these strains (Table S1), which still share 319 unique genes among each other. The phylogenomic analysis indicated a high genetic exchange between strain O3.65^T and on 16S basis more distantly related organisms [15]. Taken together, strain O3.65^T cannot be classified into the existing genera within the *Rhodobacteraceae*, indicating that strain O3.65^T represents a new species of a new genus. The multitude of reclassifications of species especially within the *Phaeobacter-Leisingera* cluster [17, 42] and *Ruegeria* [45] shows the difficulty of accurate classification of (new) species among the closely related genera within this group.

Ubiquinone Q10, which is generally dominant in bacteria of the *Roseobacter* group [17], was the sole respiratory lipoquinone present in strain O3.65^T. The polar lipids of strain O3.65^T comprised phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid, two unidentified phospholipids and several unidentified lipids (Fig. S1). The polar lipid composition differed from related type strains of *Phaeobacter*, *Leisingera* and *Ruegeria* [17] as strain O3.65^T

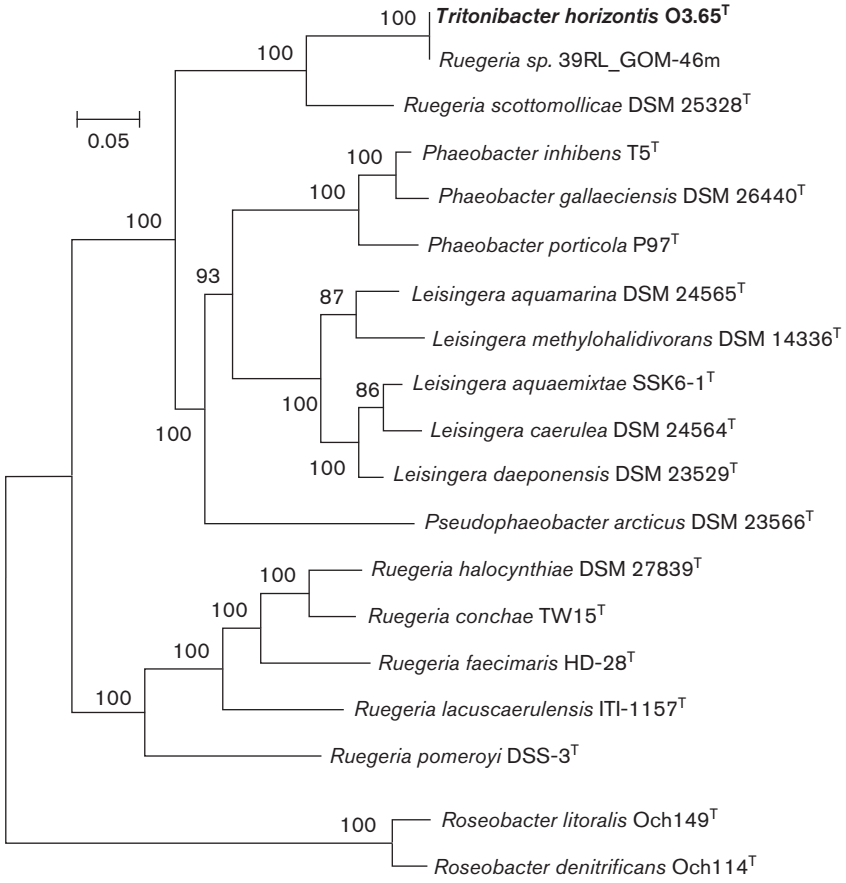

Fig. 3. Maximum-likelihood tree showing the phylogenetic position of strain *Tritonibacter horizontis* O3.65^T relative to closely related type strains within the *Rhodobacteraceae* based on the phylogeny of 1475 core genes. Support values based on 1000 bootstrap replicates. Appropriate accession numbers of strains are listed in Table S1. Bar, 0.05 average amino acid substitutions per site.

did not contain phosphatidylethanolamine, but more unidentified lipids in partially high amounts (L6). The major fatty acids of strain O3.65^T were 18 : 1ω7*c* followed by 18 : 1ω7*c* 11-methyl, which are predominant in many genera within the *Alphaproteobacteria*. Strain O3.65^T differs from related genera by fatty acids that were present in lower concentrations (Table 1). O3.65^T exclusively produced 12 : 1 and an unidentified fatty acid (with an equivalent chain-length of 16.804, possibly 17 : 1). In contrast to *Phaeobacter* and *Leisingera*, O3.65^T did not produce the fatty acid 14 : 1. Furthermore, strain O3.65^T contained lower amounts of saturated fatty acids and more unsaturated hydroxyl fatty acids in comparison to *Phaeobacter* and *Leisingera* species and *Ruegeria atlantica* 1480^T.

Phenotypical and physiological characteristics of strain O3.65^T in comparison with its close relatives are compiled in Tables 2 and S2. O3.65^T differentiates from related genera by colony colour, G+C content, temperature and salinity range, and its substrate spectrum. In addition, the 16S rRNA gene sequence, the genome and fatty acid profile of O3.65^T diverge from its closest described relatives. Therefore, strain O3.65^T belongs to a new species of a new genus, for which we propose the name *Tritonibacter horizontis* gen. nov., sp. nov.

DESCRIPTION OF *TRITONIBACTER* GEN. NOV.

Tritonibacter (Tri.to.ni.bac′ter. L. n. *Triton*, Greek god of the sea, merman and son of Poseidon; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Tritonibacter*, a Tritionian rod, referring to the marine habitat and more specifically to Triton's composite upper body of a human combined with a fish tail, signifying the discrepancy in 16S rRNA gene phylogeny and genomic analysis of this novel bacterial genus).

Cells are Gram-negative, rod-shaped and motile by means of polar flagella. Catalase and oxidase positive. Requires sodium ions for growth. Aerobic and chemoorganoheterotrophic bacteria, able to utilize amino acids, carboxylic acids and sugars as well as hydroxylated aromatic hydrocarbons. The G+C content is 61 mol%. The sole respiratory lipoquinone is Q10. The polar lipids comprise phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid, two unidentified phospholipids and seven unidentified lipids. The fatty acids are dominated by 18 : 1ω7*c* followed by

Table 1. Fatty acid content (%) of strain O3.65^T and type strains of phylogenetically related genera: 1, *Tritonibacter horizontis* O3.65^T; 2, *Phaeobacter inhibens* T5^T [17]; 3, *Phaeobacter gallaeciensis* BS107^T [17]; 4, *Leisingera aquamarina* DSM 24565^T [46]; 5, *Leisingera methylohalidivorans* MB2^T [17]; 6, *Ruegeria scottomollicae* CCUG55858^T [47]; 7, *Ruegeria atlantica* 1480^T [17]; 8, *Nautella italica* LMG 24365^T [48] and 9, *Pseudophaeobacter arcticus* 20188^T [49]. TR, trace; ND, not described. Fatty acids labelled '?' were not unambiguous identified and the unknown fatty acid is indicated with equivalent chain-length (ECL).

Fatty acid	1	2	3	4	5	6	7	8	9
10 : 0	–	–	–	ND	–	ND	–	–	–
10 : 0 3OH	3.1	1.7	1.9	>1	1.8	>1	0.4	3.2	6.8
12 : 0	–	–	–	ND	–	ND	–	–	–
12 : 0 3OH	TR	1.6	1.3	>1	2.3	ND	8.7	2.4	–
12 : 1	2.5	–	–	ND	–	ND	–	–	–
14 : 0	–	–	–	ND	–	ND	3.8	–	–
14 : 1	–	2.2	2.1	ND	2.3	ND	–	ND	–
14 : 1 3OH/3-oxo-14 : 0	2.7	0.9	0.9	ND	–	ND	1.8	–	–
15 : 1ω8*c*	TR	–	–	ND	–	ND	–	–	–
16 : 0	2.6	3.8	3.8	>1	5.1	>1	6.3	1.8	9.7
16 : 0 2OH	4.4	3.1	3.9	>1	7.0	>1	10.4	5.5	4.0
16 : 1 2OH	0.7	–	–	ND	–	ND	–	–	–
16 : 1ω7*c*	0.6	TR	TR	ND	TR	ND	TR	–	–
18 : 0	0.5	3.1	2.6	ND	1.0	ND	2.3	1.4	0.5
18 : 1 2OH	1.9	–	–	ND	–	>1	–	–	0.6
18 : 1ω9*c*	–	0.8	0.9	ND	2.5	ND	–	–	–
18 : 1ω7*c*	72.8	73.8	75.5	>1	70.3	>1	45.5	74.5	44.6
18 : 1ω7*c* 11-methyl	6.8	7.5	6.6	ND	6.9	>1	30.4	4.5	18.1
Unknown (ECL 11.799)	–	–	–	>1	–	>1	–	–	–
Unknown (ECL 16.804)	0.9	–	–	ND	–	ND	–	–	–
?	ND	–	–	ND	–	ND	2.4	–	–
?	ND	0.6	0.6	ND	1.0	ND	2.3	–	–

18 : 1ω7*c* 11-methyl. The fatty acids (>1 %) comprise 10 : 0 3OH, 12 : 1, 12 : 1 3OH, 14 : 1 3OH/3-oxo-14 : 0, 16 : 0, 16 : 0 2OH, 18 : 1 2OH. 16S rRNA gene sequence, supported by dDDH as well as genomic analysis, demonstrate that the genus *Tritonibacter* represents a separate branch within the *Rhodobacteraceae*, with *Phaeobacter gallaeciensis* DSM 26640^T and *P. inhibens* T5^T as closest described species. The type species is *Tritonibacter horizontis*.

DESCRIPTION OF *TRITONIBACTER HORIZONTIS* SP. NOV.

Tritonibacter horizontis (ho.ri.zon'tis L. gen. n. *horizontis* of the horizon, referring to the Deep Water Horizon oil spill).

The description is identical to that of the genus, with the following additional characteristics. Colonies growing on MB plates are of light beige colour, smooth and convex with regular edges. Single cells are ovoid rods of 1.3–2.2 µm length and 0.6–1.0 µm width and able to grow on a large salinity range of 0.5–10 % NaCl, with an optimum at 3.5–4.0 %. Temperature range is between 4–45 °C, whereas growth at 4, 40 and 45 °C was very weak. Temperature optimum was at 30–35 °C. The pH-values between 5.5 to 9.0 were tolerated, the optimum was pH 7. Exoenzyme activities for amylase (starch), gelatinase (gelatin) and tweenase (Tween80) were negative. Nitrite was not reduced as electron acceptor. No resistance was detectable against ampicillin, chloramphenicol, gentamicin, kanamycin, penicillin G and streptomycin. The vitamin biotin is essential for growth of O3.65^T. Requires sodium ions for growth. Growth was detectable on the following carbohydrates as the sole carbon source: (+)-L-arabinose, (+)-cellobiose, (–)-D-fructose, (–)-L-fucose, (+)-D-galactose, (+)-D-glucosamine, (+)-D-glucose, (+)-D-mannitol, (+)-D-mannose, (–)-D-ribose, (+)-sucrose, (+)-D-xylose with the exception of (+)-lactose and starch. The genes necessary for degrading these two carbohydrates were not found in the genome. The amino acids alanine, arginine, asparagine, aspartic acid, glutamic acid, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine enabled growth, but not cysteine, glutamine, glycine, lysine and methionine. Among the aromatic acids p-coumarin, 3,4-dihydroxy-benzoic acid, 4-hydroxy-benzoic acid, ferulic acid, and vanillin were used for growth, but not benzoic acid, cinnamic acid and salicylic acid. None of the alkanes nonane, decane, hexadecane or paraffin enabled growth. In addition, citrate, sodium acetate, sodium pyruvate, glycerol and DMSO were used for growth, but DMSP not. No growth was observed on oil and paraffin containing agar plates.

The type strain, O3.65^T (=DSM 101689^T=LMG 29740^T), was isolated from oil-contaminated surface seawater during

Table 2. Differential characteristics of strain O3.65^T compared to type strains of other genera: 1, *Tritonibacter horizontis* O3.65^T [15]; 2, *Phaeobacter inhibens* T5^T [17]; 3, *Phaeobacter gallaeciensis* BS107^T [17, 50]; 4, *Leisingera aquamarina* DSM 24565^T [46]; 5, *Leisingera methylohalidivorans* MB2^T [17, 46, 51, 52]; 6, *Ruegeria scottomollicae* CCUG55858^T [47]; 7, *Ruegeria atlantica* 1480^T [53, 54] and 8, *Nautella italica* LMG 24365^T [48]; 9, *Pseudophaeobacter arcticus* 20188^T [49]. All strains compared here are catalase and oxidase positive. +, positive; –, negative; ND, not determined; W, weak.

Characteristic	1	2	3	4	5	6	7	8	9
DNA G+C content (mol%)	61.5[a]	55.7	57.6 –58[b]	61.4[b]	60.5[b]	61.0[b]	59.7[b]	61[a]	59.6[a]
Similarity of 16S rRNA gene sequence to O3.65^T (%)	100	98.3	98.3	98.0	97.9	98.1	96.1	96.9	96.3
Colony colour	White-beige	Brown	Brown	Dark beige-pink	No pigment	Beige	Colourless, beige	Beige	Yellow
Temperature range (°C)	4–45	4–36	15–37	4–37	15–36	4–40	5–30	4–45	0–24
NaCl (%)	0.5–10	1.2–4	0.6–11.7	1–7	1–6	1–15	3–10	1–5	2–8
Nitrate reduction	–*	–	–	–	–	–	+	–	–
Fatty acids:									
12 : 1	+*	–	–	ND	–	ND	–	–	–
14 : 1	–*	+	+	ND	+	ND	–	ND	–
unkown (ECL 16.804)	+*	–	–	ND	–	ND	–	–	–
Motility	+	+	+	+	+	+	–	+	+
TDA production	–	+	+	–	–	–	–	–	–
Vitamin requirements	Biotin*	None	Thiamine	None	None	ND	ND	ND	ND

*This study; *a*, laboratory data; *b*, genomic data.

the Deepwater Horizon oil spill in the Gulf of Mexico. The G+C content of the type strain is 61.5 mol%.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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