Planktomarina temperata gen. nov., sp. nov., belonging to the globally distributed RCA cluster of the marine Roseobacter clade, isolated from the German Wadden Sea

Helge-Ansgar Giebel,1 Daniela Kalhoefer,1 Renate Gahl-Janssen,1 Yoe-Jin Choo,2 Kiyoungh Lee,2 Jang-Cheon Cho,2 Brian J. Tindall,3 Erhard Rhiel,1 Christine Beardsley,1 Ömer O. Aydogmus,1 Sonja Vogt,4 Rolf Daniel,4 Meinhard Simon1 and Thorsten Brinkhoff1

1Institute for Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, D-26111 Oldenburg, Germany
2Division of Biology and Ocean Sciences, Inha University, Incheon, Republic of Korea
3Leibniz Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, D-38124 Braunschweig, Germany
4Institute of Microbiology & Genetics, Genomic & Applied Microbiology and Göttingen Genomics Laboratory, University of Göttingen, D-37077 Göttingen, Germany

Four heterotrophic bacterial strains belonging to the globally distributed marine RCA (Roseobacter clade-affiliated) cluster (family Rhodobacteraceae, class Alphaproteobacteria) were obtained from coastal seawater samples. Strain RCA23T was isolated from a 10^-7 dilution culture inoculated with seawater from the German Wadden Sea (southern North Sea), reflecting the high abundance of RCA bacteria in this habitat. Strains IMCC1909, IMCC1923 and IMCC1933 were isolated from diluted seawater (10^-3) of the Yellow Sea, South Korea. Based on 16S rRNA gene sequence comparison, Octadecabacter antarcticus 307T is the closest described relative of the RCA strains, with 95.4–95.5% sequence similarity. Cells of RCA23T, IMCC1909, IMCC1923 and IMCC1933 are small motile rods requiring sodium ions. Optimal growth of RCA23T occurs at 25 °C and within a very narrow pH range (pH 7–8, optimum pH 7.5). The DNA G+C base content of RCA23T is 53.67 mol%. The major respiratory lipoquinone is ubiquinone-10 (Q-10) and the dominant fatty acids (>1%) are 12:1 3-OH, 16:1 ω7c, 16:0, 18:1 ω7c, 18:0 and 11-methyl 18:1 ω7c. The polar lipid pattern indicated the presence of phosphatidylglycerol, two unidentified aminolipids and two unidentified phospholipids. On marine agar, RCA23T forms non-pigmented, transparent to light beige, small (1 mm), circular, convex colonies. Strain RCA23T harbours all genes for the production of bacteriochlorophyll a (BChl a). Genes encoding the light-harvesting reaction centre of BChl a (pufM) were identified in all RCA strains. No visible pigmentation was observed for any of the strains under laboratory conditions, but spectrophotometric analysis revealed weak production of BChl a by RCA23T. Morphological, physiological and genotypic features of strain RCA23T suggest that it represents a novel species of a new genus within the Rhodobacteraceae, for which we propose the name Planktomarina temperata gen. nov., sp. nov., described previously by Giebel et al. [ISME J 5 (2011), 8–19] as ‘Candidatus Planktomarina temperata’. The type strain of Planktomarina temperata is RCA23T (=DSM 22400T=JCM 18269T).

Abbreviations: AAnP, aerobic anoxygenic photosynthesis; BChl, bacteriochlorophyll; DGGE, denaturing gradient gel electrophoresis; ITS, intergenic transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains RCA23T, IMCC1909, IMCC1923, IMCC1933 and HTCC2150 are GQ369962, GQ468662–GQ468664 and NZ_AAXZ00000000, respectively.

A supplementary table and two supplementary figures are available with the online version of this paper.

053249 © 2013 IUMS Printed in Great Britain 4207
Bacteria of the *Roseobacter* clade within the class *Alphaproteobacteria* have been detected in many marine habitats, often at high abundance (Buchan *et al.*, 2005). They gain energy from a multitude of organic compounds, and some are capable of aerobic anoxygenic photosynthesis (AAnP) by harvesting light energy via bacteriochlorophyll *a* (BChla) without CO₂ fixation (e.g. Brinkhoff *et al.*, 2008). None of them is able to grow autotrophically. The RCA (*Roseobacter* clade-affiliated) cluster (also called the NAC11-3 or DC5-80-3 cluster) consists primarily of 16S rRNA gene sequences of uncultured organisms from planktonic habitats and is the largest cluster of the *Roseobacter* clade (Buchan *et al.*, 2005). One organism of this cluster, strain LE17, was isolated from Californian coastal waters (Mayali *et al.*, 2008), but no strain has yet been comprehensively characterized physiologically. Distribution of members of the RCA cluster is restricted to temperate and polar water masses (Selje *et al.*, 2004; Rusch *et al.*, 2007; Giebel *et al.*, 2009), suggesting adaptation to colder habitats. The RCA cluster constitutes up to 35% of the total bacterioplankton, with the highest proportions in the Southern Ocean (Selje *et al.*, 2004; West *et al.*, 2008; Giebel *et al.*, 2009) and proportions of up to 20% in temperate and Arctic regions (Malmstrom *et al.*, 2007; Giebel *et al.*, 2011). In the German Wadden Sea (southern North Sea), 16S rRNA gene signatures of members of the RCA clade were persistently detected in environmental samples and in dilution cultures, constituting ~5–20% of the total bacterial community (Selje & Simon, 2003; Selje *et al.*, 2004, 2005; Stevens *et al.*, 2005; Giebel *et al.*, 2011).

We were able to obtain several isolates of the RCA cluster from the temperate zone. Strain RCA23ᵀ was isolated from the German Wadden Sea using dilution cultures. In a previous study, we already described strain RCA23ᵀ as ‘*Candidatus Planktomarina temperata*’ (Giebel *et al.*, 2011). Further, we isolated three RCA strains from the Yellow Sea, which shares a number of properties with the southern North Sea, as a coastal sea with extended intertidal flat areas (Otto *et al.*, 1990; Lü *et al.*, 2007). However, no data on the abundance of the RCA cluster are available for the Yellow Sea.

In this study, isolate RCA23ᵀ was characterized. Comparison of physiological and genetic capacities of the novel strain with three Korean RCA strains (IMCC1909, IMCC1923 and IMCC1933), strain LE17 (Mayali *et al.*, 2008; 99% 16S rRNA gene sequence similarity) and strain HTCC2150 (~96% 16S rRNA gene sequence similarity), isolated from the coast of Oregon, USA (Kang *et al.*, 2010), indicated that HTCC2150 probably belongs to a different genus from the other strains.

Even though isolation of strains of the RCA cluster was successful, their cultivation and physiological characterization were difficult or impossible with routinely used microbiological methods for physiological and biochemical characterization. Therefore, we chose a number of less commonly used approaches (e.g. HPLC, fluorescent substrate analogues for hydrolytic enzyme activities) in the field of descriptive microbiology in order to investigate the metabolic characteristics of strain RCA23ᵀ as reliably as possible. Hence, this study is an example for a difficult but possible description of an important member of the marine bacterioplankton community.

Strain RCA23ᵀ was isolated from a 10⁻⁷ dilution of a most-probable-number series with autoclaved seawater as medium, amended with thiosulfate (final concentration 10 mM), a trace element solution and a vitamin solution (both described by Schut *et al.*, 1993). The dilution series was inoculated with surface seawater of the German Wadden Sea collected during high tide near Neuharlinger Siel, Germany (53° 42′ N 07° 43′ E), and incubated for 7 weeks in the dark at 15°C. Dilution steps from 10⁻³ to 10⁻⁹ were screened using a PCR specific for 16S rRNA genes of RCA bacteria (Selje *et al.*, 2004) and the presence of other bacteria was analysed by a combination of PCR and denaturing gradient gel electrophoresis (DGGE) specific for the domain *Bacteria* (Teske *et al.*, 2000). All RCA-positive dilution steps were transferred to the same seawater medium. After 60 days of incubation at 15°C in the dark, cultures were screened again by PCR and DGGE. In five of the cultures, the presence of only one organism, affiliated with the RCA cluster, was indicated by DGGE. Aliquots of these cultures were transferred to autoclaved seawater, amended with trace element solution and vitamins (Schut *et al.*, 1993) and 10% marine broth (MB 2216; Difco Becton Dickinson), as well as to the medium containing thiosulfate. Subsequently, growth was tested with different MB concentrations (10–80% in 10-fold increments) at 15°C in the dark, and measured as optical density (see below). Only cultures of strain RCA23ᵀ grown on medium containing 40% MB showed stable and reliable growth and hence were used for further investigations. For long-term storage, 0.5 ml culture volume of strain RCA23ᵀ was mixed with 0.75 ml sterile 88% glycerol and maintained at ~80°C. Further physiological experiments were inoculated with pre-cultures from these glycerol cultures. Purity of the isolate was checked after each transfer by light and epifluorescence microscopy after staining with SybrGreen I (Lunau *et al.*, 2005), by DGGE specific for the domain *Bacteria* (Teske *et al.*, 2000), sequencing of PCR-amplified 16S rRNA genes obtained with *Bacteria*-specific primers (Brinkhoff & Muyzer, 1997) and colony formation after spotting droplets of cell suspensions on agar plates.

Strains IMCC1909, IMCC1923 and IMCC1933 were isolated on agar plates with R2A medium made with seawater (Difco Becton Dickinson), inoculated with a serially diluted (10⁻³) surface seawater sample collected off the coast of Deokjeok Island, Yellow Sea, Korea (37° 10′ 54″ N 126° 09′ 45″ E). The agar plates were incubated aerobically at 20°C for 1 month. Colonies were transferred several times to MB plates for purification. Subsequently, pure isolates were identified by sequencing of the 16S rRNA gene.
For comparison, the RCA strain LE17 and *Rhodobacteraceae* strain HTCC2150 were considered. These strains were provided by Xavier Mayali (Scripps Institution of Oceanography, San Diego, CA, USA) and Steven Giovannoni (Oregon State University, Corvallis, OR, USA), respectively. Strain LE17 was isolated from a bloom of the dinoflagellate *Lingulodinium polyedrum* in surface waters at Scripps Pier, La Jolla, CA, USA (32° 52.02’ N 117° 15.44’ W) in summer 2005, as described in detail by Mayali et al. (2008), and was grown in 40 % MB. Strain HTCC2150 was isolated from coastal Pacific Ocean surface seawater off Newport, OR, USA (44° 12.32’ N 124° 24.7’ W; Kang et al., 2010), applying the dilution-to-extinction culturing (high-throughput culturing) method described previously by Connon & Giovannoni (2002). Different MB concentrations for cultivation of strain HTCC2150 were tested as described for strain RCA23T. Best growth occurred when the amount of peptone and yeast extract was reduced to 0.75 and 0.14 g l⁻¹, respectively, which is equivalent to 15 % MB. The same supplements mentioned above were used. At lower and higher MB concentrations, growth of strain HTCC2150 was restricted, possibly by the presence of insufficient amounts of substrate, or was inhibited.

Cultivation on agar plates containing the above-mentioned medium was performed by spotting drops of cell suspension on the agar, because transfer from plate to plate by traditional streak-plating was often not successful, especially for strain RCA23T. Subsequent transfer to fresh agar plates resulted in much weaker and unreliable growth, so that growth on agar plates was only used to check the purity of cultures.

All tests that required liquid cultures were performed in 22.5 ml metal-capped test tubes containing 5 ml medium; autoclaved seawater supplemented with 2 g peptone 1⁻¹ (Difco) and 0.4 g yeast extract 1⁻¹ (Difco) as carbon and nitrogen sources (equivalent to 40 % MB medium and subsequently referred to as 40 % seawater MB medium), trace element solution and vitamins (Schüt et al., 1993), or in artificial seawater (ASW, with identical sea salt composition to MB 2216) amended with the same supplements as for autoclaved seawater medium, subsequently referred to as 40 % ASW MB medium. Equivalent to 40 % MB medium, we also used 6 or 15 % MB, seawater MB and ASW MB medium for growth tests (with 6 % MB medium, only very weak growth was observed). If not stated otherwise, all tests were performed in triplicate and with appropriate negative controls. Growth in liquid medium was determined by an increase in OD₆₀₀ measured with a spectrophotometer (Spectronic 70; Bausch & Lomb) or by epifluorescence microscopy after staining with SybrGreen I (Lunau et al., 2005).

The temperature range for growth was examined between 4 and 35 °C at intervals of 5 °C in the dark in 40 % seawater MB medium. The pH range for growth was tested at pH 4–10, in increments of 0.5 pH units, in 40 % seawater MB medium adjusted with sterile HCl and NaOH (1 M each). Incubation was done at 15 °C in the dark. During incubation, the pH of the medium was monitored, and no significant spontaneous changes were observed. To determine the range of osmotolerance of the isolate, the following salt concentrations were prepared: 0, 3, 6, 15, 20, 30, 35, 40, 50, 55 and 60 g salts l⁻¹, using MB diluted with deionized water or amended with a concentrated NaCl solution (3 M) as appropriate.

Anaerobic growth was tested in 40 % seawater MB medium in glass tubes with airtight rubber septa under an atmosphere of N₂/CO₂ (90 : 10, v/v) in the dark at 20 °C. ‘*Desulfobacterium corrodens*’ DSM 15630 served as a positive control.

Reduction of nitrate was tested by the method of Smibert & Krieg (1994) in 40 % seawater MB medium. *Roseobacter denitrificans* DSM 7001T served as a control.

The following physiological tests were performed according to methods described by Smibert & Krieg (1994): catalase reaction, cytochrome oxidase reaction, presence of urease, hydrolysis of Tween 80 (1 %, v/v) and starch (0.2 %, w/v) and liquefaction of gelatin (0.4 % w/v) using diluted MB 2216 medium. The cell-wall structure was determined by Gram staining (Murray et al., 1994) and by cell lysis after mixing a cell suspension with 3 % (w/v) KOH (Gregersen, 1978).

Tests for hydrolytic enzyme activities were carried out with fluorogenic substrate analogues (Hoppe, 1993). Substrates for phosphatase, β-glucosidase, chitinase and aminopeptidase activity were 4-methylumbelliferyl phosphate, 4-methylumbelliferyl β-glucopyranoside, 4-methylumbelliferyl N-acetylglucosaminide and L-leucine–4-methyl-7-glucopyranoside, 4-methylumbelliferyl β-glucopyranoside, 4-methylumbelliferyl N-acetylglucosaminide and l-leucine–4-methyl-7-glucoaminylamide (Sigma), respectively. The final concentration for each substrate was 100 μM; blanks were immediately stopped using glutaraldehyde (final 1 %) and fluorescence was measured at 460 and 360 nm excitation with a microplate reader (FLUORStar Optima; BMG) after incubation for 16 h in the dark at 15 °C.

To determine the organic carbon sources for growth of strains RCA23T, IMCC1909, IMCC1923, IMCC1933 and HTCC2150, tests on single substrates (1 g l⁻¹) were performed in ASW medium, as described by Martens et al. (2006). For each substrate, three replicates were used per strain, each inoculated with cells (washed with ASW) from exponentially growing cultures of strains RCA23T, IMCC1909, IMCC1923 and IMCC1933 incubated in 40 % seawater MB medium and of strain HTCC2150 incubated in 15 % seawater MB medium. For each medium, duplicates without inoculation and inoculated but without carbon source served as negative controls. Growth was determined by measuring the OD₆₀₀ after incubation at 20 °C for 6 weeks. When no growth was observed, tests were repeated with the addition of three drops of sterile 0.1 % yeast extract to each of the test tubes, according to Wagner-Döbler et al. (2003). Even under these modified conditions, no growth was observed.
conditions, no growth of any of the strains was observed after 6 weeks of incubation.

Physiological characteristics were also tested using the substrate panel of the API 20 NE and API 50 CH systems (bioMérieux). Carbon sources of these systems were listed previously by Wagner-Döbler et al. (2003). Even using this standard system, no growth of any of the strains was observed after 6 weeks.

The utilization of single amino acids was examined by growth tests over a period of 78 days using cultures of strain RCA23T containing 6% seawater MB medium each amended with a single amino acid (L-aspartic acid, L-glutamic acid, L-asparagine, L-histidine, L-serine, L-arginine, L-glycine, L-threonine, L-β-alanine, L-alanine, γ-amino butyric acid, L-methionine, L-valine, L-phenylalanine, L-isoleucine and L-leucine; 9 mM final concentration).

Because no growth or only weak growth was observed applying standard tests in medium containing single amino acids as carbon and nitrogen sources, utilization of dissolved free amino acids and total hydrolysable dissolved amino acids (THDAA) was analysed by HPLC after dissolved free amino acids and total hydrolysable dissolved amino acids were extracted with cold acetone/methanol (7:2, v/v), as described by Lunau et al. (2006). Samples were obtained after inoculation and after 8, 14 and 21 days of incubation from triplicate cultures of strain RCA23T, grown in 6% MB medium, amended with vitamins and incubated at 15 °C in the dark. Uninoculated MB medium served as a reference control. Samples were filtered through 0.2 μm low-protein-binding filters (Tuffrin Acrodisc; Pall) and kept frozen at −20 °C until analysis. Concentrations of the following amino acids were measured: L-aspartic acid, L-glutamic acid, L-asparagine, L-histidine, L-serine, L-arginine, L-glycine, L-threonine, L-alanine, L-tyrosine, L-methionine, L-valine, L-phenylalanine, L-isoleucine and L-leucine. L-Asparagine and L-methionine were not detectable after hydrolysis.

To determine utilization of different single mono- and disaccharides, growth tests were conducted using cultures of strain RCA23T containing 6% seawater MB medium amended with (+)-l-arabinose, (+)-D-glucose, (+)-L-fucose, (+)-L-rhamnose, (+)-D-ribose, (+)-D-fructose, (+)-sucrose, (+)-trehalose, (+)-cellulobiose, (+)-maltose, (+)-lactose, (+)-D-mannose, (+)-D-galactose or (+)-D-xylose (10 mM final) as sole carbon source. A possible decrease in the concentration of dissolved free neutral carbohydrates was analysed by HPLC with a Carbopac PA 1 column (Dionex) and pulsed amperometric detection, according to Mopper et al. (1992). NaOH (18 mmol L−1) was used as eluent. Cultures without additional supplements served as a control. Incubation was done at 20 °C in the dark, whereas sampling was done after 42 and 80 days, in the same way as for amino acids. Growth of all cultures used for the HPLC approach was also monitored by measuring the OD600. Determination of amino acid and carbohydrate concentrations by HPLC was performed to substantiate uncertain results of other standard tests.

Potential growth of strain RCA23T was tested on the following organic acids (obtained from their sodium salts) in aqueous solution of 6% MB seawater medium: formate, acetate, citrate, propionate, fumarate, lactate, glutarate, acrylate, oxalate, pyruvate, malate, butyrate, succinate and valerate (final concentrations tested were 1 and 10 mM).

Osmolytic substances, i.e. putrescine, creatine, choline, creatinine, sarcosine, glycine and spermidine, were tested as single substrates for strain RCA23T (final concentrations tested were 1 and 10 mM) in 6% MB seawater background medium. Besides OD600 measurements, we monitored growth by determination of cell numbers by flow cytometry (BD Accuri C6) using SybrGreen I staining (Giebel et al., 2011).

Autoclaved seawater and 15% seawater MB medium were amended with dimethylsulfoxoniopropionate (DMSP) and with DMSO to a final concentration of 10 μM. The possible enhancement of growth by these methylated substrates was compared with growth of controls containing no methylated supplements. Cultures were incubated at 20 °C in the dark.

Susceptibility to antibiotics was determined by adding single antibiotics to cultures of strain RCA23T grown in 40% seawater MB medium at 15 °C in the dark. The following antibiotics (each at final concentrations of 1 mM, 100 μM and 10 μM) were tested: ampicillin, chloramphenicol, gentamicin, kanamycin, penicillin G and streptomycin. A culture without antibiotic supplements served as a positive control, and growth was determined by measuring the OD600.

Requirement for single vitamins by strain RCA23T was tested with mixtures of seven vitamins (riboflavin, thiamine, nicotinic acid, pyridoxamine, pantothenate, cyanocobalamin and biotin; final concentration 5 μg L−1 each) in which one of these vitamins was omitted.

Production of BChl a was determined by spectrophotometric analysis. Cells grown in the dark were harvested by centrifugation at 10 000 r.p.m. for 10 min, and pigments were extracted with cold acetone/methanol (7:2, v/v), as described by Clayton (1966). Absorbance of the extract was analysed in the range 350–1000 nm with a Specord 40 photometer (Jena Analytik). Roseobacter denitrificans DSM 7001T was used as a positive control.

Colony morphology was studied with a Leitz Aristoplan microscope and an Axiolab 2 microscope (Zeiss). Possible motility of the strains was checked by light microscopy of exponentially growing cultures. Accumulation of poly-β-hydroxybutyrate (PHB) was determined by the Sudan black staining method (Smibert & Krieg, 1994) using a Zeiss Axiolab 2 light microscope. For transmission electron microscope observations, cells were negatively stained with phosphotungstic acid or uranyl acetate (both 1%, w/v) or studied unstained as described previously (Chávez et al., 2004) in order to preserve their native shape. After air drying, the copper grids [200 mesh (Plano)] or carbon-coated...
400 mesh (Pioliform)] were examined with a model EM 902A transmission electron microscope (Zeiss).

Chemotaxonomic analyses were done after Tindall (1990a, b, 1996). Fatty acid methyl esters were released from 20 µg freeze-dried cells from the late exponential phase using methodologies which release only ester-linked fatty acids or ester- and amide-linked fatty acids (Labrenz et al., 1998; Strömpl et al., 1999).

The DNA G+C content was deduced from the genomes of strains RCA23\textsuperscript{T} (S. Voget and others, unpublished results), LE17 (http://genome.jgi-psf.org/ros1/ros1_Linfo.html) and HTCC2150 (Kang et al., 2010).

Primers 16S-27F and 16S-1492R (Lane, 1991) were used for PCR amplification of nearly complete 16S rRNA genes of the RCA strains following a standard PCR program (Allgaier et al., 2003). The intergenic transcribed spacer (ITS) region between the 16S and 23S rRNA genes was amplified using primers 16S-907F (Muyzer et al., 1998) and 23S-189R (Hunt et al., 2006) using the PCR program described by Brinkhoff & Muyzer (1997).

To amplify fragments of the \textit{psu}M gene, coding for the subunits of the reaction centre of BChl for AAnP, the primer set of Yutin et al. (2005) was used and PCR was performed as described by Giebel et al. (2011).

PCR amplicons were purified using the peqGOLD Cycle-Pure kit (S-Line) (peqlab) and subsequently sequenced by GATC Biotech (Konstanz, Germany) using the above-mentioned primers and 518R (Muyzer et al., 1998) and 1300 bp (Hunt et al., 2006) using the PCR program described by Brinkhoff & Muyzer (1997).

After 14 days at 20 °C on agar containing 40 % seawater MB medium, strain RCA23\textsuperscript{T} forms small colonies (<1 mm in diameter) that are smooth and convex, with regular edges and transparent to light beige. Older colonies become more beige. Colonies of IMCC1909 and IMCC1923 were beige and 1–5 mm in diameter after 10 days on marine R2A agar. Colonies of IMCC1933 were light beige and nearly 1 mm in diameter after 5 days on marine R2A agar. Single cells of strains RCA23\textsuperscript{T} are rods, 1.5–2.8 × 0.6–1.1 µm (Fig. 1), dividing by binary fission. Strains RCA23\textsuperscript{T}, IMCC1909, IMCC1923 and IMCC1933 were shown to have a Gram-negative cell-wall structure. No spore formation was observed. All RCA strains (RCA23\textsuperscript{T}, IMCC1909, IMCC1923, IMCC1933) showed flagellar motility during cultivation. Strains IMCC1909 and IMCC1923 showed (filamentous) agglutination during the exponential growth stage. For strain IMCC1933, this was much less pronounced. Agglutination was not observed for strain RCA23\textsuperscript{T}. Strain RCA23\textsuperscript{T} showed pleomorphic characteristics, which have also been described for other organisms of the \textit{Roseobacter} clade, e.g. \textit{Leisingera methylalcoholivorans} (Schafer et al., 2002).

Scanning and transmission electron micrographs showed cyto-inclusions for strain RCA23\textsuperscript{T} (Fig. 1). We identified these granules as PHB by Sudan black staining in strains RCA23\textsuperscript{T}, IMCC1909, IMCC1923 and IMCC1933. The physiological pathway of PHB production was detected previously in some organisms of the \textit{Roseobacter} clade (Wagner-Döbler et al., 2004; Cho & Giovannoni, 2004). Further, the genes \textit{phaC} and \textit{phaZ}, coding for a poly(\textit{R})-hydroxyalkanoic acid synthase and a polyhydroxyalkanoate depolymerase, respectively, were found in the genome of strain RCA23\textsuperscript{T} (S. Voget and others, unpublished results). Thus, the physiological observations are consistent with the genotypic information. Transmission electron micrographs of strain RCA23\textsuperscript{T} also confirmed the observed motility during cultivation by the presence of a monotrichous flagellum (Fig. 1c).

Strain RCA23\textsuperscript{T} is a strictly aerobic, non-pigmented and Na\textsuperscript{+}-requiring bacterium, with growth occurring between 1.5 and 5.0 % (w/v) NaCl and growing optimally at 3.0–3.5 % (w/v) NaCl. Strain RCA23\textsuperscript{T} grew at 10–30 °C, and the optimum was 25 °C. No growth was observed at 4 or 35 °C. Compared with the other RCA isolates, strain RCA23\textsuperscript{T} is only capable of sustainable growth in a very narrow pH range, between pH 7.0 and 8.0, with optimum growth at pH 7.5 (Table S1, available in IJSEM Online). It is interesting that the pH optimum corresponds to the annual pH range in the Wadden Sea (Stevens et al., 2005), from where strain RCA23\textsuperscript{T} was isolated, suggesting that it is highly adapted to its habitat.

Strain RCA23\textsuperscript{T} showed susceptibility to ampicillin, chloramphenicol and penicillin G at all tested concentrations, whereas kanamycin, gentamicin and streptomycin inhibited growth only slightly at 10 µM, but all antibiotics inhibited growth at a final concentration of 25 µM. We were not able to show susceptibility to antibiotics by applying the disc diffusion technique, because no reliable growth of the tested strains occurred on agar plates.

Only thiamine was required as a supplement for reliable growth of strain RCA23\textsuperscript{T}. All strains were catalase- and oxidase-positive (RCA23\textsuperscript{T} only weak). Exoenzyme activities for amylase, gelatinase, urease and lipase (Tween 80) tested on agar were negative for strains RCA23\textsuperscript{T}, HTCC2150, IMCC1909, IMCC1923 and IMCC1933; however, we again emphasize that growth of these strains on agar plates is weak and thus these results are not reliable. No nitrification activity was shown for strain RCA23\textsuperscript{T}. Tests with fluorogenic substrate analogues revealed hydrolytic enzyme activities of strain RCA23\textsuperscript{T} for phosphatase, β-glucosidase and amine/peptidase but not for chitinase.

Growth of any of the strains was observed only in media containing yeast extract and peptone. No growth or
unreliable growth was observed in tests providing single carbon and nitrogen sources in ASW or using the API systems. At the time of writing, it has not been possible to design a minimal medium for any strain of the RCA cluster. These organisms obviously need special growth factors that are provided by complex media containing yeast extract and peptone (e.g. MB medium) and/or natural seawater. Similar findings have been reported previously for other organisms of the Roseobacter clade, e.g. for Planktotalea frisia (Hahnke et al., 2012) and Oceanibulbus indolifex (Wagner-Döbler et al., 2004).

As mentioned above, after 5 weeks of incubation, no increase in OD600 was observed for cultures of strain RCA23T containing single amino acids in comparison with control cultures without supplements. In contrast, cultures containing 6% seawater MB medium amended with a single amino acid allowed weak growth of strain RCA23T on L-alanine, L-γ-aminobutyric acid, L-glutamic acid, L-phenylalanine and L-serine and not reliable growth on L-asparagine, L-threonine, L-β-alanine, L-valine and L-isoleucine. However, in cultures of strain RCA23T grown in 6% MB medium, the concentration of THDAA, determined by HPLC, decreased significantly (P<0.001) in the course of incubation relative to the controls (6% MB without strain RCA23T), i.e. all detectable single amino acids were consumed, indicating their metabolism (Fig. S1). After 21 days of incubation, concentrations of all single amino acids decreased significantly; those of L-valine and L-phenylalanine showed an increase after 14 days, but dropped again after 21 days. Only L-isoleucine and L-leucine showed their strongest decrease in concentration after 14 days; concentrations of all other single amino acids decreased over the entire incubation period or levellised off after a first decrease until the end of the experiment (L-histidine and L-tyrosine). This indicated enzymic degradation of the amino acids from the pool of particulate combined amino acids in the medium or metabolic formation and release by strain RCA23T.

In addition, growth was detected by OD600 measurements of cultures of strain RCA23T in 6% seawater MB medium amended with (+)-L-arabinose, (−)-L-fucose and (+)-L-rhamnose, and weak growth was detected with (−)-D-fructose, (+)-D-mannose, (+)-D-glucose, (−)-D-ribose, (+)-D-galactose, (−)-trehalose, (+)-cellobiose and (−)-maltose. Detected growth on (+)-L-arabinose, (+)-L-rhamnose and (+)-D-galactose was accompanied by a significant decrease of the substrate concentration measured by HPLC, illustrating the metabolism of these single monosaccharides by RCA23T (no HPLC data are available for the other monosaccharides). Unreliable growth was detected for (−)-lactose, (+)-sucrose and (−)-D-xylose.

Growth on single acids or osmolytic substances in 6% MB seawater background medium was observed for acetate, formate, pyruvate, putrescine and spermidine, whereas propionate, oxalate, malate, succinate, fumarate, valerate, butyrate, creatine and creatinine promoted only weak growth. Citrate, acrylate, glutarate and lactate resulted in unreliable growth, while choline, sarcosine and glycine yielded no detectable growth of strain RCA23T.

Supplementation of the medium with methylated substrates (DMSP, DMSO) as an additional nutrient source accelerated and enhanced growth and cell yield of cultures of strains RCA23T, IMCC1909, IMCC1923 and IMCC1933. The mean OD600 of cultures after 7 days of incubation supplemented with DMSP or DMSO was significantly higher (t-test, P<0.001) than without supplements.

Phylogenetic analyses based on 16S rRNA gene sequences demonstrated that strains RCA23T, IMCC1909, IMCC1923, IMCC1933 and LE17 form a distinct monophyletic lineage within the Roseobacter clade in the family Rhodobacteraceae (Garrity et al., 2006) of the Alphaproteobacteria, not associated with any of the currently described genera (Fig. 2). Based on data available in the GenBank database, the degree of 16S rRNA gene sequence similarity within the RCA cluster is >98% (Selje et al., 2004; Giebel et al., 2009). Sequence comparisons to the type strains of other species with validly published names indicate that strains RCA23T, IMCC1909, IMCC1923, IMCC1933 and LE17 are most closely related to Octadecabacter antarcticus 307T (GenBank accession no. U14583; 95.3–95.4% sequence similarity) and Octadecabacter arcticus 238T (U73725; 94.7–94.8% sequence similarity) (Fig. 2). The nearly complete 16S rRNA gene sequences of strains RCA23T, IMCC1909 and IMCC1923 are identical, whereas that of strain IMCC1933 differs by one
RCA23T, IMCC1909, IMCC1923 and IMCC1933. Apart from these deletions, the ITS sequences of LE17 and strain RCA23T are identical, and the ITS sequence of strain LE17 differs in nine nucleotides from those of strains IMCC1909 and IMCC1923, and in 34 nucleotides from that of strain IMCC1933. Based on 16S rRNA gene sequence comparisons, strain HTCC2150 shows at least 5.0–5.2 % sequence divergence from all RCA strains investigated in this study, and more than 9 % difference in the 16S rRNA gene plus the ITS region, whereas the heterogeneity of the 16S rRNA gene plus the ITS region is up to 6 % among the RCA strains.

The capacity for AAnP is a prominent feature of some organisms of the *Roseobacter* clade (family *Rhodobacteraceae*, class *Alphaproteobacteria*). Only 16S rRNA gene sequences >1300 bp were considered. *Methyllococcus capsulatus* ACM 1292 (GenBank accession no. X72770) and *Thiothrix nivea* JP2 (L40993) were used as an outgroup (not shown) to define the root of the tree. Filled circles indicate nodes that were also recovered reproducibly with maximum-likelihood calculation. Numbers at nodes are bootstrap values (only values >50 % are shown) from 2000 replicates. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position.

### Fig. 2

Neighbour-joining tree based on 16S rRNA gene sequence similarity showing the relationships of strain RCA23T (in bold) and selected representatives of the *Roseobacter* clade (family *Rhodobacteraceae*). Only 16S rRNA gene sequences >1300 bp were considered. *Methyllococcus capsulatus* ACM 1292 (GenBank accession no. X72770) and *Thiothrix nivea* JP2 (L40993) were used as an outgroup (not shown) to define the root of the tree. Filled circles indicate nodes that were also recovered reproducibly with maximum-likelihood calculation. Numbers at nodes are bootstrap values (only values >50 % are shown) from 2000 replicates. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position.

nucleotide. The 16S rRNA gene sequence of strain LE17 has two base deletions compared with the other four RCA strains. After resequencing, however, we could not confirm these deletions (*Escherichia coli* positions 384 and 1461) and found that the 16S rRNA gene sequences of strains LE17, RCA23T, IMCC1909 and IMCC1923 are identical. The 16S rRNA gene sequence of strain LE17 differs in one nucleotide compared with that of strain IMCC1933.

In contrast to the 16S rRNA gene, comparison of the ITS region allowed high-resolution identification of the different RCA strains. Strains IMCC1909 and IMCC1923 have identical ITS sequences, which suggests that these strains may potentially not be different. Compared with IMCC1909 and IMCC1923, strain IMCC1933 differs at 31 alignment positions and by a four-nucleotide deletion. Strain IMCC1933 differs from RCA23T in 30 nucleotide positions and by a deletion of four bases. Strain RCA23T exhibits nine nucleotide variations in its ITS sequence compared with strains IMCC1909 and IMCC1923. The ITS sequence of strain LE17 possesses 28- and 81-base insertions compared with the sequences of strains RCA23T, IMCC1909, IMCC1923 and IMCC1933. Apart from these insertions, the ITS sequences of LE17 and strain RCA23T are identical, and the ITS sequence of strain LE17 differs in nine nucleotides from those of strains IMCC1909 and IMCC1923.
pigmentation by BChl a was observed, however, for any of the strains under laboratory cultivation conditions, but spectrophotometric analysis revealed weak production of BChl a by strain RCA23T compared with R. denitrificans DSM 7001T (Fig. S2), confirming the genotypic information.

The DNA G+C content of strain RCA23T is 53.67 mol%, similar to the value for strain LE17, which has a DNA G+C content of 54.4 mol%. The DNA G+C content of strain HTCC2150, at 49.1 mol%, is clearly different from those of the RCA strains. The chemical composition of strains RCA23T and HTCC2150 confirms that ubiquinones are the sole respiratory lipoquinones present, with ubiquinone-10 (Q-10) being the major component. The dominant fatty acids (>1%) of strain RCA23T were 12:1 3-OH, 16:1ω7c, 16:0, 18:1ω7c, 18:0 and 11-methyl 18:1ω7c; 10:0, 9:0 3-OH, 12:1 and 12:0 were present at <1%. In strain HTCC2150, the fatty acids were 10:0 3-OH, 12:1, 12:0, 12:1 3-OH, 14:0, 15:0, 14:1 3-OH, 16:1ω7c, 16:0, 18:1ω7c, 18:0 and 11-methyl 18:1ω7c (Table 1). The polar lipids of strain RCA23T were phosphatidylglycerol, two unidentified aminolipids and two unidentified phospholipids, while HTCC2150 had a more complex pattern, comprising phosphatidylglycerol, one unidentified aminolipid, three unidentified phospholipids and two unidentified lipids.

The phenotypic, physiological and genetic characteristics of strain RCA23T are compared with those of its closest described relative Octadecabacter antarcticus 307T and the type species of related genera (Fig. 2) in Table 2. Characteristics of strains RCA23T, IMCC1909, IMCC1923, IMCC1933, LE17 and HTCC2150 are summarized in Table S1. Phylogenetic analysis of the 16S rRNA gene sequence as well as of the ITS region of strain HTCC2150 indicates that this strain is not affiliated with the RCA cluster (Fig. 2). The absence of genes coding for components of BChl a synthesis, the DNA G+C content and further physiological and chemotaxonomic characteristics (Tables 1 and S1) confirm this result. Thus, strain HTCC2150 might represent a species of another new genus of the Roseobacter clade. Whether the different strains of the RCA cluster belong to one species cannot be decided at present. However, our data already show that some genetic and physiological diversity exists among different organisms of the RCA cluster. Genotypic and phenotypic differences between strain RCA23T and previously described genera support the description of this strain as a member of a novel species within a new genus, for which we propose the name Planktomarina temperata gen. nov., sp. nov.

### Description of Planktomarina gen. nov.

Planktomarina (Plank.to.ma.ri’na. Gr. adj. planktos drifting, wandering; L. adj. marinus of or belonging to the sea, marine; N.L. fem. n. Planktomarina a planktonic/drifting bacterium isolated from seawater).

Cells are Gram-negative, rod-shaped and motile. Require sodium ions for growth. Cells are capable of producing PHB granules. Catalase- and oxidase-positive. Aerobic and photo-chemo-organoheterotrophic, able to utilize complex organic substrates included in MB including amino acids, carboxylic acids and sugars. Contain pufM genes encoding the reaction centre of BChl a. The DNA G+C content is approximately 54 mol%. The major respiratory lipoquinone is Q-10. The fatty acids (>1%) comprise 12:1 3-OH, 16:1ω7c, 16:0, 18:1ω7c, 18:0 and 11-methyl 18:1ω7c. The fatty acid composition is dominated by 18:1ω7c (a common feature of many members of the class Alphaproteobacteria). The fatty acids 10:0, 9:0 3-OH, 12:1 and 12:0 are found in traces (<1% each). The polar lipids comprise phosphatidylglycerol, two unidentified aminolipids and two unidentified phospholipids. 16S rRNA gene sequence analysis demonstrates that the genus represents a separate branch (probably equivalent to the RCA cluster) within the Roseobacter clade (Rhodobacteraceae), with Octadecabacter antarcticus 307T as the closest described relative. The type species is Planktomarina temperata.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>M1</th>
<th>M2</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0</td>
<td>0.4</td>
<td>0.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9:0 3-OH</td>
<td>0.8</td>
<td>0.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10:0 3-OH</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>1.7</td>
<td>2</td>
<td>3.6</td>
<td>3.3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>12:1</td>
<td>0.6</td>
<td>0.6</td>
<td>2.5</td>
<td>2.1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12:0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12:1 3-OH</td>
<td>2.3</td>
<td>4.8</td>
<td>1.7</td>
<td>1.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>14:0</td>
<td>--</td>
<td>--</td>
<td>0.2</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>15:0</td>
<td>--</td>
<td>--</td>
<td>0.2</td>
<td>0.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>14:1 3-OH</td>
<td>--</td>
<td>--</td>
<td>2.5</td>
<td>4.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>16:1ω7c</td>
<td>12.6</td>
<td>12.2</td>
<td>2.8</td>
<td>2.7</td>
<td>12</td>
<td>1.2</td>
<td>--</td>
<td>--</td>
<td>0.4</td>
</tr>
<tr>
<td>16:0</td>
<td>9.0</td>
<td>8.7</td>
<td>4.7</td>
<td>4.7</td>
<td>6</td>
<td>8.1</td>
<td>1.5</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>18:1ω7c</td>
<td>59.7</td>
<td>58.2</td>
<td>83.0</td>
<td>83.0</td>
<td>77*</td>
<td>79.1</td>
<td>91.8</td>
<td>86.4</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>2.1</td>
<td>2.1</td>
<td>0.7</td>
<td>0.7</td>
<td>--</td>
<td>--</td>
<td>1.6</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>11-Methyl</td>
<td>11.1</td>
<td>10.9</td>
<td>1.5</td>
<td>1.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.6</td>
</tr>
<tr>
<td>18:1ω12t</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*Unclear whether the component detected was 18:1ω7c, 18:1ω9t or 18:1ω12t.
**Table 2.** Differential characteristics of strain RCA23<sup>T</sup> and its closest related and described phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA gene sequence similarity to RCA23&lt;sup&gt;T&lt;/sup&gt; (%)*</td>
<td>(100)</td>
<td>95.3</td>
<td>94.6</td>
<td>94.6</td>
</tr>
<tr>
<td>DNA G+G content (mol%)</td>
<td>53.67†</td>
<td>56</td>
<td>62.0–62.5</td>
<td>57.2</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Small, transparent to light beige, circular, convex</td>
<td>White, circular, convex, entire</td>
<td>Colourless, flat</td>
<td>Pink</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod or ovoid rod</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>0.6–1.1 × 1.5–2.8</td>
<td>0.6–0.8 × 1.6–4.8</td>
<td>0.45–1.3 × 2–5</td>
<td>0.6–0.9 × 1.2–2.0</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>10–30</td>
<td>4–10</td>
<td>4–35</td>
<td>2–30</td>
</tr>
<tr>
<td>Optimum</td>
<td>25</td>
<td>nd</td>
<td>22–25</td>
<td>20–30</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>7–8</td>
<td>6.5–9.5</td>
<td>6.5–8.5</td>
<td>7.5–9.5</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>BChl a</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pufM gene</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>nd</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>nd</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Alanine</td>
<td>w</td>
<td>nd</td>
<td>+</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>w</td>
<td>nd</td>
<td>+</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>nd</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetate</td>
<td>+</td>
<td>–</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propionate</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>nd</td>
</tr>
<tr>
<td>Butyrate</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>w</td>
<td>w</td>
<td>w&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>w</td>
<td>–</td>
<td>nd</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>–&lt;sup&gt;$&lt;/sup&gt;</td>
<td>–</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–&lt;sup&gt;$&lt;/sup&gt;</td>
<td>–</td>
<td>–&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
</tr>
</tbody>
</table>

* Determined by distance matrix of the neighbour-joining tool of the ARB software package.
† Data from genome sequencing.
§ Tested on agar plates, where growth of the strains is restricted.
|| Arahal et al. (2005).

**Description of Planktomarina temperata sp. nov.**

*Planktomarina temperata* (tem.pe.ra’ta. L. fem. part. adj. temperata moderate, referring to the occurrence of the species at latitudes with moderate temperature).

The description is identical to that of the genus, with the following additional properties. Cells are 1.5–2.8 µm long and 0.6–1.1 µm wide. Colonies on agar are small (<1 mm in diameter), smooth and convex, with regular edges, and are transparent to light beige after more than 14 days of incubation at 20 °C. Older colonies become more beige. Cells grow at 10–30 °C, with optimum growth at around 25 °C, and at pH 7–8 (optimum pH 7.5). Cells grow only in media containing natural seawater and complex supplements (peptone and yeast extract). Halotolerance ranges from 1.5 to ~5.0 % (w/v) NaCl [optimum 3–3.5 % (w/v) NaCl]. Negative for amylase, gelatinase and Tweenase. Photosynthetic growth is not observed reliably under laboratory conditions; however, the entire BChl<sub>a</sub> operon for aerobic anoxygenic photosynthesis is found in the genome. Slight production of BChl<sub>a</sub> pigments is detected. Not able to reduce nitrate to nitrogen. Weak chemo-organoheterotrophic growth on different single compounds requiring a background medium containing complex substances such as MB. Optimum growth on complex media with natural seawater containing essential...
but so far unknown growth factors. The following substrates are utilized in 6% seawater MB medium: (+)-L-arabinose, (-)-L-fucose, (+)-L-rhamnose, acetate, formate, pyruvate, putrescine, spermidine, DMSP and DMSO; weak growth on (-)-d-fructose, (+)-d-mannose, (+)-d-glucose, (-)-d-ribose, (+)-d-galactose, (+)-trehalose, (+)-cellobiose, (+)-maltose, L-alanine, β-aminoallyl acid, L-glutamic acid, L-phenylalanine, L-serine, propionate, oxalate, malate, succinate, fumarate, valerate, butyrate, creatine and creatine-nine. HPLC analyses support utilization of (+)-L-arabinose, (+)-L-rhamnose, (+)-d-galactose, L-glutamic acid, L-alanine, L-serine and L-phenylalanine, and enhance the spectrum of utilized amino acids to L-glycine, L-aspartate, L-histidine, L-arginine, L-threonine, L-tyrosine, L-valine, L-isoleucine and L-leucine. Requires thiamine for growth. Susceptible to ampicillin, chloramphenicol and penicillin G (10 µM final concentration) and to kanamycin, gentamicin and streptomycin (25 µM final concentration).

The type strain, RCA23T (=DSM 22400T =JCM 18269T), was isolated from surface water of the German Wadden Sea, southern North Sea. The DNA G+C content of the type strain is 53.67 mol%.

Acknowledgements

We thank Andrea Schlingloff, Birgit Kürzel, Mathias Wolterink, Rolf Weinert and Michael Pilzen for technical assistance. We are most grateful to the Oregon State University High Throughput Culturing Laboratory, headed by Steven Giovannoni, and to Farooq Azam and Xavier Mayali for providing strains HTCC2150 and LE17, respectively. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) within the Special Priority Program on Antarctic Research, the Transregional Collaborative Research Center (TRR-51) and the 21C Frontier Program of Microbial Genomics and Applications funded by MEST, Republic of Korea.

References


Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors


