Rhodothermus obamensis sp. nov., a Modern Lineage of Extremely Thermophilic Marine Bacteria

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A novel extremely thermophilic bacterium was isolated from a shallow marine hydrothermal vent environment (depth, 22 m) in Tachibana Bay, Nagasaki Prefecture, Japan. The cells of this organism were gram-negative rods. Growth occurred at temperatures between 50 and 85°C (optimum temperature, 80°C; doubling time at optimum temperature, 90 min), at pH 5.5 and 9.0 (optimum pH, 7.0), and in the presence of 1 and 5% NaCl (optimum NaCl concentration, 3%). The new isolate was an aerobic heterotroph which utilized the following compounds as sole energy and carbon sources: yeast extract, peptone, starch, casein, Casamino Acids, a variety of sugars, some carboxylic acids, and amino acids. As determined by a sequence analysis of the 16S rRNA, the new isolate belongs to the genus *Rhodothermus* and represents a modern lineage of extreme thermophiles within the domain *Bacteria*. On the basis of the physiological and molecular properties of the new isolate, we describe a new species, *Rhodothermus obamensis*. The type strain of *R. obamensis* is strain OKD7 (= JCM 9785).

Over the last 10 years, a number of new genera and species of thermophilic organisms which are capable of growth at temperatures up to 80°C have been isolated. Most of these organisms belong to the domain *Archaea* (31), but there are a few genera, such as the genera *Thermus*, *Thermotoga*, and *Aquifex*, which belong to the domain *Bacteria* (5, 10, 11). These thermophiles have distinct physiological differences; the members of the genus *Thermus* are strictly aerobic heterotrophs, the members of the genus *Thermotoga* are strictly anaerobic heterotrophs, and the members of the genus *Aquifex* are microaerobic chemolithoautotrophs. On the basis of 16S rRNA analysis data, the genera *Thermotoga* and *Aquifex* represent the deepest phylogenetic branches in the domain *Bacteria* (7, 21). Moreover, the genus *Thermus* also branches deeply on the phylogenetic tree inferred from 16S rRNA sequences (21). These findings and the results of a phylogenetic study of the thermophilic genus *Hydrogenobacter* support the hypothesis that the ancestors of bacteria were thermophilic species (1, 26).

The members of the genus *Rhodothermus*, on the other hand, are marine thermophilic bacteria that have been isolated from sites in Iceland and the Azores (2, 20). *Rhodothermus marinus* is an aerobic heterotrophic bacterium that has an optimum growth temperature of 65°C and has been used in studies of genetic engineering of thermostable enzymes (28). A 16S rRNA analysis of this organism placed the genus *Rhodothermus* close to the root of the *Flexibacter-Cytophaga-Bacteroides* group with affinities to the green sulfur bacteria, fibrobacteria, and spirochetes (3). This phylogenetic position of the genus *Rhodothermus* differs from the phylogenetic positions of other thermophilic bacteria and suggests that there are thermophilic or extremely thermophilic bacteria which are distant from the universal root of life and that there was another origin of thermophily within the *Bacteria* (3).

In this paper, we describe the isolation and characterization of a new extremely thermophilic *Rhodothermus* species that

was obtained from a shallow marine hydrothermal vent and discuss the importance of the phylogenetic position of this organism to the hypothesis that the origin of thermophily within the *Bacteria* is ancient.

MATERIALS AND METHODS

Collection of samples. Effluent water samples from hydrothermal vents and sedimentary materials adjacent to vents were collected from Tachibana Bay, Nagasaki Prefecture, Japan, at a depth of 22 m. The temperatures of the hydrothermal vent fluids were 120 to 125°C. Samples were stored aerobically at room temperature for 6 h prior to incubation.

Enrichment and purification. Subsamples of effluent water and sediment samples were used to inoculate a series of media, including Jx medium (see below). All of the Jx medium tubes containing sediment were turbid after 1 day of incubation at 80°C. To obtain a pure culture, enriched cells were streaked onto Jx medium plates and the plates were incubated at 70°C. Well-defined colonies were picked and streaked onto another plate, and this plate was incubated at 70°C. This procedure was repeated at least five times. A pure colony that was red was designated strain OKD7^T (T = type strain).

Culture medium and conditions. The new isolate was routinely cultivated in Jx medium, which contained (per liter) 35 g of Jamarin S synthetic seawater powder (Jamarin Laboratory, Osaka, Japan), 5 ml of Jamarin S synthetic seawater solution (Jamarin), 1 g of yeast extract (Difco Laboratories, Detroit, Mich.), and 1 g of Trypticase peptone (BBL, Cockeysville, Md.); the pH of this medium was adjusted to 6.8 to 7.2 with H₂SO₄. For growth on plates, 3% (wt/vol) GP-700 agar (Shimizu Shokuhin, Shimizu, Japan) was added to Jx medium. To determine the effect of NaCl on growth, different concentrations of an NaCl solution (pH 7.0) containing 0.1% (wt/vol) yeast extract and 0.1% (wt/vol) peptone were used instead of Jx medium. In order to determine the effects of temperature and pH on growth, the new isolate was cultivated in MJ medium, which contained (per liter) 30.0 g of NaCl; 0.14 g of K₂HPO₄; 0.14 g of CaCl₂ · 2H₂O; 0.25 g of NH₄Cl; 3.4 g of MgSO₄ · 7H₂O; 4.18 g of MgCl₂ · 6H₂O; 0.33 g of KCl; 0.5 mg of NiCl₂ · 6H₂O; 0.5 mg of Na₂SeO₃ · 5H₂O; 0.01 g of Fe(NH₄)₂(SO₄)₂ · 6H₂O; 10 ml of a trace mineral solution containing (per liter) 1.5 g of nitrilotriacetic acid, 3.0 g of MgSO₄ · 7H₂O, 0.5 g of MnSO₄ · 2H₂O, 1.0 g of NaCl, 0.1 g of FeSO₄ · 7H₂O, 0.18 g of CoSO₄ · 7H₂O, 0.1 g of CaCl₂ · 2H₂O, 0.18 g of ZnSO₄ · 7H₂O, 0.01 g of CuSO₄ · 5H₂O, 0.02 g of KAl(SO₄)₂ · 12H₂O, 0.01 g of H₃BO₃, 0.01 g of Na₂MoO₄ · 2H₂O, 0.025 g of NiCl₂ · 6H₂O, and 0.3 mg of Na₂SeO₃ · 5H₂O; 1 g of yeast extract; and 1 g of Trypticase peptone. The pH of MJ medium was adjusted to 6.8 to 7.2 with H₂SO₄ for the growth temperature test and to various values with H₂SO₄ or NaOH for the pH test. To examine the utilization of carbon-containing substrates, the yeast extract and peptone in MJ medium were replaced with various concentrations of substrates.

Light microscopy. Cells were routinely observed with a differential interference microscope (Nikon). For microscopy at 80°C, 1 drop of a hot culture was placed on a slide that had been preheated to about 85°C and was observed

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immediately. Micrographs were taken with a Nikon optishot microscope equipped with a Nikon FX-II camera system.

Determination of growth. Growth of the new isolate was determined by measuring turbidity at 660 nm and by direct cell counting after preparations were stained with 4',6-diamidino-2-phenylindole. Duplicate cultures were grown in 300-ml flasks containing 100 ml of medium in a temperature-controlled dry oven (Advantec, Tokyo, Japan) and were shaken at 100 rpm. The growth curves under changing pH and NaCl concentration conditions were determined at 75°C.

Organic substrates for growth. To find organic substrates which supported growth of the new isolate, experiments were conducted in MJ medium containing potential substrates in place of yeast extract and peptone. The potential substrates were added at concentrations of 0.01, 0.05, and 0.1% (wt/vol). Cells were precultured in each medium and inoculated into the same medium. Duplicate tests were performed at 75°C.

Cellular fatty acid and quinone compositions. Cellular fatty acid and quinone compositions were determined by using OKD7^T cells cultivated in Jx medium at 75°C. The cellular fatty acid content was determined as described by Kawasumi et al. (14), while the major quinone content was determined as described by Ishii et al. (12).

Isolation and base composition of DNA. DNA was prepared as described by Marmur and Doty (19) and Lauerer et al. (18). The G+C content of the DNA was determined by directly analyzing the deoxyribonucleotides by high-performance liquid chromatography (27). A DNA GC kit (Yamasa, Chiba, Japan) was used as the standard.

Amplification of 16S rRNA gene and sequence determination. The 16S rRNA gene was amplified by the PCR. The sequences of the primers used for amplification were 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-GGTTACCTTCC TCCGGCTTA-3', corresponding to positions 8 to 27 and 1492 to 1511, respectively, in the 16S rRNA sequence of *Escherichia coli* (6). The 1.5-kb PCR product was directly sequenced by the dideoxynucleotide chain termination method by using a model 373As DNA sequencer (Applied Biosystems, Inc.). The DNA sequence data were used in a sequence homology search with previously described 16S rRNA sequences by using DNASIS software (Hitachi Software, Tokyo, Japan).

Data analysis. The almost complete 16S rRNA sequence of OKD7^T was aligned with other sequences, and the evolutionary distances (K_{nuc} values) were calculated by using 1,407 nucleotides (corresponding to position 29 to position 1436 on the *E. coli* 16S rRNA sequence). The ODEN version 1.1.1 software package (National Institute of Genetics, Mishima, Japan) was used to align multiple sequences, to calculate the K_{nuc} values, and to construct a phylogenetic tree based on the results of the neighbor-joining method (24) and a bootstrap examination.

Nucleotide sequence accession number. The almost complete 16S rRNA sequence of strain OKD7^T has been deposited in the EMBL nucleotide sequence database under accession number X95071.

RESULTS

Enrichment and purification. Enrichment took place from sedimentary materials in Jx medium at 80°C, and a mixed population of coccoid and long, thin, rod-shaped cells were observed after 1 day of incubation. A culture containing rod-shaped cells was purified during repeated plating at 70°C. A single red colony was used as the inoculum for a liquid culture that was designated strain OKD7^T (= JCM 9785^T [Japan Collection of Microorganisms, Wako, Japan]). All experiments were performed with isolate OKD7^T.

Morphology. Cells of OKD7^T were gram-negative rods which were 4 to 10 µm long and 0.5 to 1.0 µm wide in the exponential growth phase (Fig. 1A). In the stationary phase, the cells tended to occur in aggregates (Fig. 1B) and to become extremely long (Fig. 1C). Isolate OKD7 grew vigorously with shaking and was a typical aerobe.

Temperature range for growth and effects of pH and salt concentration. The new isolate grew at temperatures of about 50 to 85°C; the optimum temperature was 80°C, and the generation time at 80°C was about 1.5 h (Fig. 2A). No growth occurred at 90°C. Growth of the new isolate occurred at pH values between pH 5.5 and 9.0, and the optimum pH was about 7.0 (Fig. 2B). No significant change in pH was observed during growth, and no growth was detected at pH values below 5.5 or above 9.0.

Strain OKD7^T required NaCl for growth. It grew in the presence of about 1 to 5% NaCl, and the optimum NaCl

FIG. 1. Differential interference micrographs of *R. obamensis* cells. (A) Exponentially growing cells. (B) Aggregated cells in the stationary phase. (C) Filamentous cells in the stationary phase. Bars = 5 µm.

FIG. 2. Effects of temperature, pH, and NaCl on the growth of *R. obamensis*. (A) Temperature range for growth of *R. obamensis* in MJ medium having an initial pH of 6.8 to 7.2. No growth occurred at temperatures below 50°C and above 85°C. (B) pH range for growth of *R. obamensis* in MJ medium at 75°C. The initial pH was adjusted with H₂SO₄ or NaOH at room temperature. (C) Effect of NaCl on growth of *R. obamensis* at 75°C. For this experiment, solutions containing different concentrations of NaCl, 0.1% (wt/vol) yeast extract, and peptone (pH 7.0) were used.

concentration was around 3% NaCl (Fig. 2C). At NaCl concentrations below 1% or above 5%, the cells lysed within 2 h.

Nutrition. Strain OKD7^T was a heterotroph. In MJ medium containing 0.1% (wt/vol) yeast extract and 0.1% (wt/vol) peptone, OKD7^T grew vigorously with a generation time of about 1.5 h and reached a maximum cell density of more than 10⁹ cells per ml. Similar growth rates and somewhat lower maximum cell densities were observed when 0.1% (wt/vol) yeast extract, peptone, and Casamino Acids were used as sole energy and carbon sources, whereas with sucrose, mannitol, sorbitol, or starch the growth rates and cell yields were quite similar to the growth rates and cell yields with 0.1% (wt/vol) yeast extract and peptone (Table 1). The new isolate also utilized a variety of sugars, amino acids, and other compounds as substrates for growth (Table 1). Very weak growth occurred in the presence of valine, and no growth occurred in the presence of xylose, glycerol, glutamine, or leucine (Table 1). In addition, high concentrations of carbohydrates inhibited growth, as shown for glucose (Table 1 and data not shown). In contrast, the growth rates and cell yields increased with the concentrations of sub-

strates such as carboxylic acids, amino acids, Casamino Acids, and casein (Table 1 and data not shown).

Fatty acid and quinone compositions. When the new isolate was grown at 75°C, the major cellular fatty acids were iso-C₁₇ acid (39%), anteiso-C₁₅ acid (19%), anteiso-C₁₇ acid (16%), and iso-C₁₅ acid (15%). This fatty acid composition was similar to the fatty acid composition of *Thermus* spp. (Table 2). However, there was a significant difference between the quinone composition of OKD7^T and the quinone composition of *Thermus* spp. Menaquinone 8 is the major menaquinone of *Thermus* spp., but menaquinone 7 is the major respiratory quinone (83%), and menaquinones 5 and 6 are also present (7 and 10%, respectively); however, no menaquinone 8 was detected in OKD7^T (Table 2).

DNA base composition. The G+C content of OKD7^T was 66.6 mol%, a value similar to the values obtained for *R. marinus* and *Thermus* spp. (Table 2).

Phylogenetic analyses. The almost complete 16S rRNA sequence of OKD7^T was determined and was found to be 95% similar to the 16S rRNA sequence of *R. marinus* but less than 80% similar to any other sequences. This result suggested that the new isolate belongs to the genus *Rhodothermus*. Therefore,

TABLE 1. Maximum cell yields and growth rates for *R. obamensis* OKD7^T with various carbon-containing substrates^a

Substrate(s)	Specific growth rate (%) ^b	Maximum cell density (cells/ml)
Yeast extract (0.1%) + peptone (0.1%)	100	2 × 10 ⁹
Yeast extract (0.1%)	89	1 × 10 ⁹
Peptone (0.1%)	96	1 × 10 ⁹
Glucose (0.01%)	64	1 × 10 ⁸
Glucose (0.05%)	22	7 × 10 ⁷
Glucose (0.1%)	7	4 × 10 ⁷
Galactose (0.01%)	66	2 × 10 ⁸
Maltose (0.01%)	45	1 × 10 ⁸
Sucrose (0.01%)	86	5 × 10 ⁸
Cellobiose (0.01%)	70	2 × 10 ⁸
Mannitol (0.01%)	77	4 × 10 ⁸
Sorbitol (0.01%)	80	2 × 10 ⁸
Xylose (0.01%)	0	
Starch (0.01%)	80	5 × 10 ⁸
Lactate (0.1%)	28	8 × 10 ⁷
Pyruvate (0.01%)	5	4 × 10 ⁷
Pyruvate (0.05%)	44	8 × 10 ⁷
Pyruvate (0.1%)	72	2 × 10 ⁸
Glycerol (0.01%)	0	
Glycerol (0.1%)	0	
Casein (0.1%)	65	2 × 10 ⁸
Casamino Acids (0.1%)	95	7 × 10 ⁸
Glycine (0.1%)	44	9 × 10 ⁷
Alanine (0.1%)	52	1 × 10 ⁸
Leucine (0.01%)	0	
Leucine (0.1%)	0	
Serine (0.1%)	33	8 × 10 ⁷
Threonine (0.1%)	35	9 × 10 ⁷
Valine (0.01%)	27	6 × 10 ⁷
Valine (0.1%)	0	
Proline (0.1%)	25	9 × 10 ⁷
Arginine (0.1%)	44	8 × 10 ⁷
Aspartate (0.1%)	48	1 × 10 ⁸
Glutamine (0.01%)	0	
Glutamine (0.1%)	0	

^a All preparations were incubated at 75°C with shaking at 100 rpm, and the initial pH of each medium was adjusted to 6.8 to 7.2 with H₂SO₄ or NaOH.

^b The specific growth rate in the medium containing 0.1% yeast extract and 0.1% peptone was defined as 100%.

TABLE 2. Comparison of some characteristics of *R. obamensis*, *R. marinus*, and the genera *Thermus* and *Thermomicrobium*

Characteristic	<i>R. obamensis</i>	<i>R. marinus</i> ^a	<i>Thermus</i> ^b	<i>Thermomicrobium</i> ^c
Cell length (µm)	4-10	2-2.5	5-10	3-6
Colony color(s)	Reddish	Reddish	Yellow, orange, red, transparent	Red
Optimum temp (°C)	80	65	70-75	70-75
Generation time (min)	90	80	20-60	330
Growth in the presence of:				
0% NaCl	—	—	+	+
3% NaCl	+	+	—	—
6% NaCl	—	+	—	—
Growth on:				
Xylose	—	+	—	NR ^d
Sorbitol	+	—	—	NR
Casein	+	—	+	+
Casamino Acids	+	—	—	NR
Glutamine	—	+	Variable	NR
Proline	+	—	+	NR
Serine	+	—	Variable	NR
Oxidase	+	+	+	NR
Catalase	+	+	+	NR
G+C content (mol%)	66.6	65	57-65	NR
Major fatty acid(s)	iso-C ₁₅ , iso-C ₁₇ , anteiso-C ₁₅ , anteiso-C ₁₇	NR	iso-C ₁₅ , iso-C ₁₇	Internally branched 12-methyl-C ₁₈
Major isoprenolog(s)	MK-7, MK-6, MK-5	NR	MK-8	NR

^a Data from references 2 and 20.^b Data from references 4, 5, and 20.^c Data from references 2 and 13.^d NR, not reported.

in order to determine the phylogenetic position of the genus *Rhodothermus*, including OKD7^T, evolutionary distances based on the 16S rRNA gene sequences of several members of the *Bacteria* and *Archaea* were calculated and a phylogenetic tree was reconstructed by using the neighbor-joining method (Fig. 3).

On the phylogenetic tree, new isolate OKD7^T was located

along with *R. marinus* close to the root of the *Flexibacter-Cytophaga-Bacteroides* group and did not represent a deep branch within the domain *Bacteria*, which was consistent with the phylogenetic position of *R. marinus* determined previously (3). The bootstrap confidence data supported the robustness and significance of the placement of OKD7^T and *R. marinus*.

DISCUSSION

The new isolate was a gram-negative, rod-shaped, aerobic extreme thermophile and appeared to be a member of the domain *Bacteria* because of its morphological and physiological similarities to other thermophilic bacteria. Representatives of thermophilic genera belonging to this domain have been isolated from various hydrothermal vents and terrestrial hot springs (2, 5, 10, 11, 13, 17, 20). On the basis of physiological properties, the new isolate resembled members of three extremely thermophilic genera, the genera *Thermus*, *Thermomicrobium*, and *Rhodothermus* (Table 2). However, the new isolate was significantly different from members of the genera *Thermus* and *Thermomicrobium* as determined by chemotaxonomic characteristics. Although the major fatty acid components of the new isolate were similar to those of *Thermus* strains the new isolate contained menaquinone 7 as its major isoprenolog; this menaquinone not only is different from the major menaquinone of *Thermus* strains (Table 2) but also is rare among the gram-negative bacteria (8). When the major cellular fatty acids were compared, *Thermomicrobium* strains had high levels of internally branched fatty acids, primarily 12-methyl C₁₈ fatty acid (4, 13), which were quite different in their branching patterns and chain lengths from the predominant fatty acids of the new isolate (Table 2).

The finding that the 16S rRNA sequence of the new isolate exhibited 95% homology with the sequence of *R. marinus* suggests that the new isolate belongs to the genus *Rhodothermus*.

FIG. 3. Phylogenetic tree showing the positions of representative members of the *Bacteria* and *Archaea* inferred from 16S rRNA sequence data. The thick lines indicate hyperthermophilic or extremely thermophilic branches and possible thermophilic origins of life. The numbers are the bootstrap values for the branches (based on 100 replicates). Scale bar = 0.1 substitution per nucleotide. The GenBank nucleotide sequence accession numbers for the organisms used in the analysis are as follows: *Sulfolobus solfataricus*, X03235; *Aquifex pyrophilus*, M83548; *Thermotoga maritima*, M21774; *Thermomicrobium roseum*, M34115; *Thermus thermophilus*, X07998; *Bacillus stearothermophilus*, X57309; *Anabaena* sp., X59559; *Escherichia coli*, V00348; *Chlorobium vibrioforme*, M62791; *Rhodothermus obamensis*, X95071; *Rhodothermus marinus*, X80994; *Theronema lapsum*, L11703; *Flavobacterium breve*, M59052; and *Cytophaga hutchinsonii*, M58768.

The members of the genus *Rhodothermus* are aerobic, heterotrophic thermophiles that have been isolated recently from sites in Iceland and the Azores (2, 20). *R. marinus* is a marine thermophile which grows at temperatures between 54 and 77°C (optimum temperature, 65°C) and utilizes a variety of sugars and carboxylic acids as sole carbon and energy sources (2, 20). In contrast, the new isolate is an extreme thermophile that grows at temperatures up to 85°C, has an optimum temperature of 80°C (Fig. 2), and utilizes different carbon-containing substrates for growth (Table 2). Unlike *R. marinus*, the new isolate utilizes sorbitol, casein, Casamino Acids, proline, and serine and does not utilize xylose or glutamine as a sole carbon and energy source (Table 2). Moreover, the morphology and salt tolerance of OKD7^T and *R. marinus* are different and the evolutionary distance between these taxa based on 16S rRNA sequence data is equivalent to the distances used to distinguish species within the domain *Bacteria*. On the basis of the results described above, we propose that the new isolate is a member of a new species of the genus *Rhodothermus*, *Rhodothermus obamensis*. The type strain of *R. obamensis* is strain OKD7.

On the basis of its optimal growth in the presence of 3% NaCl, *R. obamensis* is a typical marine organism similar to *R. marinus*. It was thought previously that members of the genus *Thermus* are the most common heterotrophs in submarine hot springs, as well as in terrestrial hot springs (15, 16). However, members of at least two heterotrophic genera in the *Bacteria*, the genera *Thermotoga* and *Rhodothermus*, have been isolated from submarine hydrothermal environments (2, 10, 20), and it has also been suggested that there are uncultivable heterotrophic thermophiles (22). The isolation of a member of the genus *Rhodothermus* from Japan submarine hydrothermal vents leads to the speculation that this genus may be widely distributed in submarine hydrothermal environments.

The results of the phylogenetic analysis of the 16S rRNA sequence demonstrated the significance of the phylogeny of the genus *Rhodothermus* on the bacterial phylogenetic tree (Fig. 3). Interestingly, both of the *Rhodothermus* species are most closely related to the members of *Flexibacter-Cytophaga-Bacteroides* group. The results of recent phylogenetic studies of thermophilic members of the *Bacteria* and *Archaea* have suggested that the deep branches on the universal tree are occupied by thermophiles and have implied that the common ancestor of all present-day organisms might be a thermophile (1, 7, 11, 26, 29, 30). Nevertheless, there has been some evidence against this hypothesis that has been based on the phylogeny of other proteins (23, 25) and the evolution of the reverse gyrase gene (9). In addition, the members of a group of thermophiles (the genera *Rhodothermus* and *Thermoneema*) placed close to the root of the *Flexibacter-Cytophaga-Bacteroides* group are not deep branches (3; this study), and there are diverse thermophilic members of the *Bacteria* that are only distantly related to the universal root (4). The discovery of the extreme thermophile *R. obamensis*, whose lineage is apparently modern, suggests that extreme thermophily is not necessarily an ancient characteristic but may have a second, modern origin within the *Bacteria*.

Description of *Rhodothermus obamensis* Sako, Takai, Ishida, Uchida, and Katayama sp. nov. *Rhodothermus obamensis* (o.bam'en.sis. L. gen. n. *obamensis*, of Obama, a hot spring in Japan). Cells are gram-negative, straight rods that are about 4 to 10 µm long and 0.5 to 1.0 µm wide and occur singly and in aggregates; they are extremely long during the stationary growth phase. Colonies are reddish and 1 to 2 mm in diameter with entire edges. Aerobic, thermophilic, neutrophilic heterotroph. Growth occurs at temperatures between 50 and 85°C (optimum temperature, 80°C), at pH 5.5 and 9.0 (optimum pH,

7.0), and in the presence of 1 to 5% NaCl (optimum NaCl concentration, 3%). Oxidase and catalase positive. The major cellular fatty acid components are anteiso- and iso-C₁₇ and anteiso- and iso-C₁₅ acids. The major quinone is menaquinone 7; smaller amounts of menaquinones 6 and 5 are also present. Growth occurs on complex medium containing yeast extract and peptone and on medium containing glucose, galactose, sucrose, maltose, cellobiose, sorbitol, mannitol, starch, lactate, pyruvate, casein, Casamino Acids, arginine, aspartate, glycine, alanine, serine, threonine, or proline. The DNA base composition is 66.6 mol%. The 16S rRNA of *R. obamensis* is 95% similar to *R. marinus* 16S rRNA.

The type strain is *R. obamensis* OKD7 (= JCM 9785), which was isolated from a site in Tachibana Bay, Nagasaki, Japan.

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