Roseovarius bejariae sp. nov. - a moderately halophilic bacterium isolated from a hypersaline steep-sided river bed

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Running title:

Roseovarius bejariae sp. nov.

Keywords: *Roseovarius bejariae* sp. nov.; polyphasic taxonomy; halophilic bacteria; saline soil

Subject category: New Taxa, **Subsection:** *Proteobacteria*

The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain A21^T is MK933766.

The genome of strain A21^T has been deposited in NCBI genome database under the accession number SZWE00000000.

Five supplementary figures are available as online Supplementary Material.

Abstract

An aerobic, gram-stain-negative ovoid, designated as strain A21^T, was isolated using the dilution-to-extinction method from a soil sample taken from Rambla Salada, an athalassohaline habitat located in Murcia (south-eastern Spain). Strain A21^T is non-motile, has a respiratory metabolism and grows at NaCl concentrations within the range 0.5–15 % (w/v) [optimum 5 % (w/v)], at 5–35ºC (optimum 28ºC) and at pH 6–8 (optimum pH 7.0). This strain is positive for catalase, oxidase and nitrate reduction. The 16S rRNA gene sequence indicates that it belongs to the genus *Roseovarius* in the class *Alphaproteobacteria*. The most closely related species are *R. pacificus and R. halotolerans* to which the strain A21^T shows a 16S rRNA gene-sequence similarity value of 98.06 % and 97.7% respectively. The average nucleotide identity in blast (ANIb) and *in silico* DNA-DNA hybridization (dDDH) between strain A21^T and *R. pacificus* LMG 24575^T were 76.8% and 21%, respectively. The DNA G+C content based on the genome was 61.28 mol %. The major fatty acids (>5% of the total fatty acids) of strain A21^T are C18:1 w7c/C18:1 w6c and $C_{16:0}$. The only detected isoprenoid quinone of strain A21^T is ubiquinone 10 (Q-10). The polar lipid profile contains phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and three unidentified polar lipids. Based on the phylogenetic, genotypic, phenotypic and chemotaxonomic data, the strain represents a novel species of the genus *Roseovarius*, for which the name *Roseovarius bejariae* sp. nov. is proposed. The strain A21^T (= CECT 9817^T= LMG 31311^T) is the type strain.

The genus *Roseovarius* was established by Labrenz et al. [1] and belongs to the family *Rhodobacteraceae* within the class *Alphaproteobacteria.*

Representative bacteria of the genus *Roseovarius* are gram-stain-negative, aerobic, ovoid or rod shaped, bacteriochlorophyll a (BChla) positive, oxidase and catalase positive and require Na^+ for growth. Most of the bacteria of this genus have been isolated from marine environments including tidal flat, hypersaline lake, deep-sea sediment or seawater [2, 3, 4, 5, 6, 7, 8]. In general, the major polar lipids representative of this genus are phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) and the major fatty acids are $C_{18:1}$ *w7c*/$C_{18:1}$ *w6c* , $C_{16:0}$ and 11-Methyl-C_{18}:1 *w7c*. At the time of writing, the genus comprises 22 published species (http://www.bacterio.net/roseovarius.html).

In this study, we report a polyphasic characterization to describe the strain $A21^T$, a moderately halophilic bacterium isolated from a saline soil in Rambla Salada (Murcia), an athalassohaline habitat located in Murcia (south-eastern Spain). The salinity of this protected habitat is mainly due to the presence of ions Na^+, Cl^-, SO_4^{2-}, and Ca^{+2}. The bacterial community of Rambla Salada has been widely studied [9] and five novel halophilic bacterial species has been described so far in this habitat. One of these bacteria was recently described as *Roseovarius ramblicola* [7]. During the course of a study focused on the isolation of new halophilic bacterial species in this area, a new strain, $A21^T$ was isolated from soil. Based on a polyphasic characterization that include phenotypic, chemotaxonomic and phylogenetic analysis, we propose in this paper to establish for this isolate the new species *Roseovarius bejariae* sp. nov.

Strain $A21^T$ was isolated from a saline soil taken from Rambla Salada (Murcia), south-eastern Spain, 38°07'27.1"N 1°07'01.4"W. The sample was collected in January 2015 using a sterile polycarbonate tube and taken immediately to the laboratory, where it was stored at 4°C until study. The salinity of the sample was around 40 g L^{-1} and the pH close to neutrality. The isolation medium utilised was S3, a low-nutrient medium [10] supplemented with 3% (w/v) sea-salt solution [11] and the cultivation method used was the dilution-to-extinction approach described previously by Castro et al [7]. The dilution-to-extinction approach is a technique that improves the isolation of slow-growing species or apparently uncultivable species [12, 13, 14]. The extinction cultures were incubated at 25ºC for 30 days and after the content of wells were then re-isolated in R2A medium plates [15]. The isolated strain was maintained and grown routinely in R2A [15] at 30ºC with 3% (w/v) sea-salt solution [11] as well as in Marine Agar (MA: 2216 Difco™).

Genomic DNA was extracted from an overnight 5 ml culture of $A21^T$ strain in R2A medium using an X-DNA purification kit (Xtrem Biotech S.L., Granada, Spain). The 16S rRNA sequence was extracted from the genome sequence and the 16S rRNA gene was also amplified by polymerase chain reaction (PCR) using the universal bacterial primers 16F27 and 16R1488 [16]. The PCR product was purified and cloned into the pGEM®-T vector (Promega). Direct sequencing of PCR-

amplified DNA was determined using an ABI PRISM DyeTerminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) and an ABI PRISM 377 sequencer (Perkin-Elmer) according to the manufacturer's instructions.

Phylogenetic analyses based on the 16S rRNA gene were performed as described elsewhere [7, 17]. The identification of phylogenetic neighbours was carried out by three methods: by using the BLASTN [18] program against the GenBank/EMBL/DDBJ database containing type strains with validly published prokaryotic names and representatives of uncultured phylotypes; by using the LTP ARB database "All-species Living Tree Project", current release (June 2018: LTPs132_SSU) [19] and by the identification of phylogenetic neighbours and a calculation of pairwise 16S rRNA gene sequence similarity by using the "EzBiocloud" server (www.ezbiocloud.net; [20]). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7 [21]. Clustering was determined using the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms and the evolutionary distances were computed using the Jukes-Cantor method [22]. The analysis involved 36 nucleotide sequences and the stability of the clusters was ascertained by performing a bootstrap analysis (1000 replications).
These analyses indicated that strain A21^T fell within the clade comprising the type strains of species of the genus *Roseovarius.* The tree containing the phylogroup in which the strain is included according to the maximum-likelihood algorithm is shown in Figure 1. The most closely related species were *R. pacificus and R. halotolerans* to which the strain A21^T shows a 16S rRNA gene-sequence similarity value of 98.06 % and 97.7%, respectively. The gene-sequence similarity with *R. tolerans*, the type strain of the *Roseovarius* genus is 95.75%.

The DNA G+C content based on the genome sequence was 61.28 mol %, a similar value to members of the genus *Roseovarius* [1, 6, 23] (Table 1).

The genome of strain A21^T was sequenced using Illumina Hi-Seq sequencing platform at the installations of STAB VIDA, Caparica, Portugal, with 2 x 150-bp paired-end reads. The reads were processed with bbduk (https://sourceforge.net/projects/bbmap/) to remove adapters and low-quality bases and reads. Then, the reads were assembled *de novo* using SPAdes v. 3.11.1 [24]. After RAST annotation of the genome, it was manually mined to find the gene annotated as "SSU rRNA 16S rRNA, small subunit ribosomal RNA", for which only one copy was found. The ANIb calculation of A21^T with *R. pacificus* LMG 24575^T and *R. halotolerans* KCTC 22224^T was carried out used JSpecies web server [25], whereas digital hybridization (DDH) was calculated using the BLAST+ algorithm in the Genome-to-Genome Distance Calculator (GGDC 2.1) of DSMZ [26]. The results here presented are based on the recommended formula 2 (Identities/HSP length).

The draft genome assembly of strain A21^T consisted in 3 contigs, with coverage of 350X and a total size of 3,498,676 bases. The genome was annotated using PGAP and RAST [27, 28] and it consisted

in 3327 total CDs, with 3 rRNAs and 21 tRNAs. The predicted proteins were further assigned with a COG category using the EggNOG tool [29]. 2929 proteins (88%) were assigned to a COG category. This data is shown in Table S1. It is remarkable that the most abundant category, besides proteins with unknown function, is category E, "amino acid transport and metabolism", followed by category C, "energy production and conversion". The average nucleotide identity in blast (ANIb) and *in silico* DNA-DNA hybridization (dDDH) between strain A21^T and *R. pacificus* LMG 24575^T were 76.8% and 21%, respectively, whereas the ANIb and dDDH between strain A21^T and *R. halotolerans* KCTC 22224^T were 76.02% and 20.1%, respectively. These values are below the proposed cut-off for the delimitation of species, which is 95-96% for ANIb and 70% for the DNA-DNA hybridization [30, 31] thus demonstrating that the strain A21^T is a novel species of the *Roseovarius* genus. The G+C content based on the genome was 61.28 mol %. Using the whole genome sequence of A21^T, a whole phylogenomic tree based on the Neighbor-Joining method was constructed using the species of *Roseovarius* for which their genome was available. To carry out this analysis, the software BPGA was used [32]. This tree is shown in Figure S1. To complete the genomic analysis, whole genome comparison was carried out between the strain A21^T and the species *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T; the closest species based on the phylogenomic tree (Fig. S2).

Phenotypic analysis was carried out in order to characterize the strain A21^T. The optimum salt growth conditions for the strain A21^T were evaluated in R2A medium added with different NaCl concentrations: 0, 0.5, 1, 3, 5, 7.5, 10, 15, 20, 25 and 30% (w/v). The optimal (and range) growth pH of A21^T was evaluated by growing the strain under different pH values (4, 5, 6, 7, 8, 9, 10 and 11). This pH values were reached using the following buffer systems: 0.1 M citric acid/0.1 M sodium citrate (pH 4.0–5.0,); 0.1 M KH_2PO_4/0.1 M NaOH (pH 6.0–8.0); 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 (pH 9.0–10.0); 0.2 M KH_2PO_4/0.1 M NaOH (pH 11.0) [33]. The effect of different temperatures (0, 5, 15, 20, 25, 28, 30, 32, 35, 40 and 45ºC) was also assessed in Marine Broth (MB, Difco™).

Gram staining was performed according to the method described by Komagata [34] and growth under anaerobic conditions was determined in an anaerobic jar using AnaeroGen (Oxoid) and an anaerobic indicator (Oxoid) using Marine Agar. Motility was observed using log phase culture according to the hanging drop method [35]. Oxidase activity was determined with 1% (v/v) tetramethyl-p-phenylenediamine and catalase activity was examined by bubble production with 3% (v/v) H_2O_2 solution [36]. Phenotypic characterization, including biochemical characters, sugar fermentation and enzymatic tests were carried out by using classical systems, API 20NE, API 50CH, API ZYM strips (bioMérieux) and Generation-III microplates (BIOLOG Inc., Hayward, CA, USA). Activity of urease and hydrolysis of DNA, casein and gelatin and the utilization of D-maltose, D-trehalose, D-cellobiose, D-raffinose, α-D-lactose, D-salicin, α-D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, D-sorbitol, D-mannitol, myo-inositol, glycerol, L-alanine, L-histidine, L-

serine, D-gluconic acid, citric acid, D-malic acid, propionic acid, acetic acid and formic acid were studied by following the method described previously by our research group [35].

The Generation-III microplates were inoculated with a cell suspension at a cell density of 95% transmittance using a turbidimeter Biolog-21907 and dye IF-A. The further additive was sea-salt solution (2% v/v). The plates were sealed with parafilm to avoid a loss of fluid.

Sensitivity to antimicrobial compounds was assayed according to the conventional Kirby-Bauer method [37] with a NaCl concentration of 30 g L^{-1} using antibiotic discs (BD Sensi-Disc™) (the quantities are expressed as µg per disc). Scanning electron micrographs of strain A21^T was realised in a FIB-FESEM (CrossBeam NVision 40®, Carl Zeiss SMT) Scanning Electron Microscope and are available as supplementary material (Fig. S3).

The cells of strain A21^T were non-motile, ovoid and gram-stain- negative. The strain can grow at 0.5–15% (w/v) NaCl, optimally at 5% (w/v) NaCl. The temperature range for growth was 5–35ºC, with the optimum being 28ºC. The pH range for growth was 6–8 with an optimum growth at pH 7.0. The strain is catalase, oxidase and nitrate reductase positive, aerobic and has a respiratory metabolism. It is susceptible to amoxicillin (25), amoxicillin/clavulanic acid (30), ampicillin (10), azithromycin (15), aztreonam (30), carbenicillin (100), cefotaxime (30), cephalotin (30), cefuroxime (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicine (10), nalidixic acid (30), rifampicin (2), penicillin (10), norfloxacin (10), and streptomycin (10) and is resistant to polymyxin B (300), tetracyclin (30), amikacin (30), neomycin (30), trimethoprim/sulfamethoxazole (23.75/1.25), sulphonamides (300), cefoxitin (30), bacitracin (10) and tobramycin (10). Other characteristics of strain A21^T are given in the species description and those that differ with the most related species and with the type species of the genus *Roseovarius* are shown in Table 1.

The fatty acids of strain A21^T were analysed at the Spanish Type Culture Collection (CECT). Cells were grown on MA for 48 h incubation at 30ºC (stationary growth phase). The whole-cell composition of the fatty acids was determined by GC using the MIDI Microbial Identification System [38]. The fatty- acid profile was obtained with an Agilent 6850 gas chromatograph using the database TSBA6 145 [39]. Analysis of polar lipids and respiratory quinones of strain A21^T was carried out by the Identification Service of DSMZ, Braunschweig, Germany. Polar lipids are extracted following the protocol described elsewhere [40]. Polar lipids are separated by two dimensional silica gel thin layer chromatography (Macherey-Nagel Art. No. 818135). The first direction is developed in chloroform:methanol:water (65:25:4, v/v/v), and the second in chloroform:methanol:acetic acid:water (80:12:15:4, v/v/v/v). Total lipid material was detected using molybdatophosphoric acid and specific functional groups detected using spray reagents specific for

defined functional groups [41]. The two stage method described by [42, 43] is used to first extract the respiratory lipoquinones followed by the polar lipids.

The major fatty acids (>5% of the total fatty acids) of strain A21^T are C18:1 w7c/C18:1 w6c (77.5%) and $C_{16:0}$ (11.9%). The fatty-acid profile of strain A21^T was similar to the profile described for *R. pacificus* LMG 24575^T, *R. halotolerans* KCTC 22224^T and *R. tolerans* EL-172^T (Table 2).

The only isoprenoid quinone detected was Ubiquinone Q10, this data is in accordance with the lipoquinone present in the described species of the genus *Roseovarius*. The major polar lipids components: phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) are similar in strain A21^T and in the type strain *R. tolerans* EL-172^T [1] (Fig. S4). However, their polar lipid profiles were distinguishable mainly in that A21^T had no diphosphatidylglycerol (DPG), and three unidentified polar lipids (L) whereas *R. tolerans* EL-172^T and *R. halotolerans* KCTC 22224^T had DPG [4, 44]. Therefore, the polar lipid composition of A21^T is quite different to the composition previously described for other members of the family *Rhodobacteraceae* such *Roseobacter litoralis* or *Marinovum algicola* [45].

To analyze the presence of bacteriochlorophyll *a*, *puf* genes (photosynthetic reaction centre genes *pufL* and *pufM*) were amplified by PCR using *puf*Lf (5′-CTKTTCGACTTCTGGGTSGG-3′) and *pufM*r (5′-CATSGTCCAGCGCCAGAA-3') as the pair of primers, following the protocol described by [46]. Supplementary Figure S5 shows the absence of *pufLM* genes in the strain A21^T and the absence of these genes in *Roseovarius pacificus* LMG 24575^T.

The combined results obtained from the phenotypic and chemotaxonomic analyses and genetic distinctiveness, revealed that strain A21^T should be recognized as representing a novel species of the genus *Roseovarius*, for which we propose the name *Roseovarius bejariae.*

Description of *Roseovarius bejariae* sp. nov.

Roseovarius bejariae (be.ja´ri.ae N.L. fem. gen. n. *bejariae* from Béjar, named in honour of professor Victoria Béjar, as a tribute to the contributions of her studies on halophilic bacteria).

Cells are ovoid, gram-negative-staining, non-motile, 0.7 x 1.6 μm in size. Cells have no flagellum. Cell colonies are pink coloured when grown on MA and R2A media, circular, convex and opaque. The growth pattern is uniform in a liquid medium. They are capable of growing in NaCl concentrations of 0.5 to 15% (w/v), with optimum growth occurring at 5%. They grow within a temperature range of 5 to 35ºC at pH values of between 6 and 8, the optimum values being 28ºC and pH 7. It is aerobic and has a respiratory metabolism. Catalase, oxidase are produced and reduction of nitrate is positive. Hydrolysis of arginine, aesculin, DNA, casein and gelatine are negative. Indol and

urease are not produced. Activity of valine arylamidase is positive, but the activities of esterase C4, esterase lipase C8, naphthol-AS-BI-phosphohydrolase, acid and alkaline phosphatase, lipase C14, leucine arylamidase, cystine arylamidase, trypsine, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative.

The strain was negative for the utilization of dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl-β-glucosamine, N-acetyl-neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, L-fucose, D-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-serine, pectine, glycyl-L-proline, trisodium citrate, adipic acid, phenylacetic acid, capric acid, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, glucuronamide, mucid acid, quinic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, methyl pyruvate, citric acid, α-keto-glutaric acid, bromo-succinic acid, α-hydroxy-butiric acid and formic acid. The strain was positive for the utilization of glycerol, D-aspartic acid, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, Tween 40, γ-amino-butyric acid, β-hydroxy-D,L-butyric acid, acetoacetic acid, propionic acid and acetic acid.

Acid production from glycerol was positive and negative from D-glucose, L-arabinose, D-maltose, D-trehalose, D-cellobiose, gentiobiose, D-turanose, D-raffinose D-melibiose, D-salicin, D-mannose, D-fructose, D-galactose L-fucose, D-fucose, L-rhamnose D-sorbitol, D- mannitol, D-arabitol.

The only detected lipoquinone is ubiquinone with ten isoprene units (Q10). The DNA G+C content of the type strain based on the genome sequence is 61.28 mol%. The major fatty acids (>5% of the total fatty acids) of strain A21^T are C18:1 w7c/C18:1 w6c and $C_{16:0}$. Bacteriochlorophyll *a* is not produced. The polar lipids contain phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and three unidentified polar lipids. The type strain A21^T (=CECT 9817^T=LMG 31311^T), was isolated from a saline soil from Rambla Salada (Murcia), south-eastern Spain, 38°07'27.1"N 1°07'01.4"W.

The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain *Roseovarius bejariae* A21^T is MK933766, and the complete genome is deposited under the accession number SZWE00000000.

Funding information

This research was supported by grants from Dirección General de Investigación Científica y Técnica (DI-14-06868; CGL2011-25748), Spain.

Acknowledgements

D.C. and I.S. wish to thank MINECO (Ministerio de Economia y Competitividad) for their "Industrial Doctorate" and "Ramón y Cajal" contracts, respectively. J.C.R. is supported by an FPU fellowship (FPU15/01717) from the Spanish Ministry of Education, Culture and Sport. We thank Mohamed Tassi Mzanzi for his expertise in the electron-microscope studies.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Table 1: Characteristics that distinguish strain A21^T from type strains of the genus *Roseovarius*. **1**, strain A21^T; **2**, *R. pacificus* LMG 24575^T; **3,** *R. halotolerans* KCTC 22224^T ;**4,** *R. tolerans* EL-172^T All strains are gram-stain-negative ovoid. All are positive for catalase and oxidase. All are negative for indole production, aesculin, DNA, casein and gelatin hydrolysis; and activity of lipase (C14), acid phosphatase, urease, trypsin, α- chymotrypsin, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, α- galactosidase, β-galactosidase, β-glucocoronidase, α- glucosidase, β–glucosidase, *N*-acetyl-β–glucosaminidase, α- mannosidase and α-fucosidase.
All are negative for the utilization of dextrin, D-maltose, D-cellobiose, gentiobiose, sucrose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, L-fucose, D-fucose, L-rhamnose, D-sorbitol, D-arabitol, myo-inositol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-serine, pectine, glycyl-L-proline, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, glucuronamide, mucid acid, quinic acid, p-hydroxy-phenylacetic acid, methyl pyruvate, bromo-succinic acid and α-hydroxy-butiric acid. All are positive for the utilization of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, D-lactic acid methyl ester, L-lactic acid, D-malic acid, L-malic acid, Tween 40, γ-amino-butyric acid, β-hydroxy-D,L-butyric acid, acetoacetic acid, propionic acid and acetic acid. D-arabinose, L-arabinose, D-maltose, D-cellobiose, gentiobiose, D-raffinose D-melibiose, D-salicin, D-mannose, D-fructose, D-galactose, L-fucose, D-fucose, L-rhamnose D-sorbitol, D-arabitol, N-acetyl-β-glucosamine, gluconate, adipic acid, phenylacetic acid, capric acid and trisodium citrate cannot be used as sole carbon source by any of the four strains.

Symbols: +, positive; -, negative; w, weak.

Characteristic	**1**	**2**	**3**	**4**
Colony pigmentation	Pink	Pink	Pink	Red
Growth ranges				
Temperature	5-35	25	10-45[a]	8.5-33.5[b]
pH	6.0-8.0	6.2-8.5	6.0-8.5	6.2- 9.0[b]
NaCl (% w/v)	0.5-15	2.0-12.0	0.5-20	1.0-8.0
Bacteriochlorophyll *a*	–	–	–	+
Nitrate reductase	+	–	–	–
Valine arylamidase	+	–	–	–
Leucine arylamidase	–	+	+	+
Alcaline phosphatase	–	+	–	–
Esterase C4	–	+	+	+
Esterase lipase C8	–	+	+	+
Acid production from:				
D-glucose	–	–	+	–
Potassium 5-ketogluconate	+	w	+	–

D-trehalose	–	–	–	+
D-turanose	–	–	–	+
D- mannitol	–	–	–	+
Glycerol	+	+	–	+
Utilization of:				
Stachyose	–	–	–	+
Inosine	–	–	–	+
D-aspartic acid	+	–	+	–
D-saccharic acid	–	+	–	–
Citric acid	–	+	+	–
α-keto-glutaric acid	–	–	+	–
α-hydroxy-butyric acid	–	+	–	–
Formic acid	–	+	–	–
DNA G+C content (mol%)	61.28 (based on genome sequence)	62.3	59[a]	62.2-63.8[b]

***Data taken from: a, [4] ; b, [1]**

554 **Table 2:** Fatty-acid profile of the strain A21^T and the type strains of the genus *Roseovarius*.
555 **1**, strain A21^T; **2**, *R. pacificus* LMG 24575^T; **3,** *R. halotolerans* KCTC 22224^T ;**4,** *R. tolerans* EL-
556 172^T Cells were grown on marine agar at 30ºC for 48 h. The major fatty acids (>5% of the total fatty
557 acids) are highlighted in bold. -, not detected. Data represent percentages of total fatty acids.
558 §Summed feature 3: $C_{16:1}$ ω7*c*/ $C_{16:1}$ ω6*c*, Summed feature 8: $C_{18:1}$ ω7*c*/ $C_{18:1}$ ω6*c*.

Fatty acids	**1**	**2**	**3**	**4**
Saturated				
C_{10}	1.1	–	–	–
$C_{12:0}$	1.6	4.2	**6.4**	–
$C_{14:0}$	0.7	–	–	–
$C_{16:0}$	**11.9**	**6.4**	**12.8**	**6.6**
$C_{16:1}$	–	–	–	0.6
$C_{17:0}$	0.6	0.3	–	–
$C_{18:0}$	1.3	0.9	3.2	0.9
$C_{18:2}$	–	–	–	**11.2**
Hydroxy				
C_{10} 3-OH	–	0.6	0.8	–
$C_{12:0}$ 2-OH	–	–	–	2.8
$C_{12:0}$ 3-OH	–	3.4	5.9	–
$C_{12:1}$ 3-OH	–	1.9	2.8	3.2
$C_{16:0}$ 2-OH	–	2.5	1.6	–
Cyclo				
$C_{19:0}$ cyclo *w8c*	–	3.6	**9.8**	–
Methyl ester				
$C_{18:1}$ *w7c* 11-methyl	3.8	**8.3**	–	–
Summed features§				
3	1.0	0.8	–	0.8
8	**77.5**	**67.2**	**56.7**	**73.9**

559

560

Figure Legend

Figure 1: Maximum-likelihood phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain A21^T, type strains of species of the genus *Roseovarius* and the closest related species of the family *Rhodobacteraceae*. Filled circles indicate nodes that were also recovered in the maximum-parsimony and neighbour-joining trees based on the same sequences. Numbers at nodes are levels of bootstrap support (percentages) based on analyses of 1000 re-sampled datasets; only values above 70% are shown. Bar, 0.05 nt changes per position. The GenBank/EMBL/DDBJ accession number of each sequence is shown in parentheses.

Abbreviations

EMBL: European Nucleotide Archive, Cambridge, UK.

DDBJ: DNA Data Bank of Japan, Mishima, Japan.

G+C: Guanine plus Cytosine content

CECT: Spanish Type Culture Collection

BCCM /LMG Belgian Coordinated Collections of Microorganisms Bacteria Collection

BChla: Bacteriochlorophyll *a*

R2A: Reasoner's 2A agar

MA: marine agar

BLAST: Basic Local Alignment Search Tool

LTP ARB: All-species Living Tree Project

MIDI: Microbial Identification System

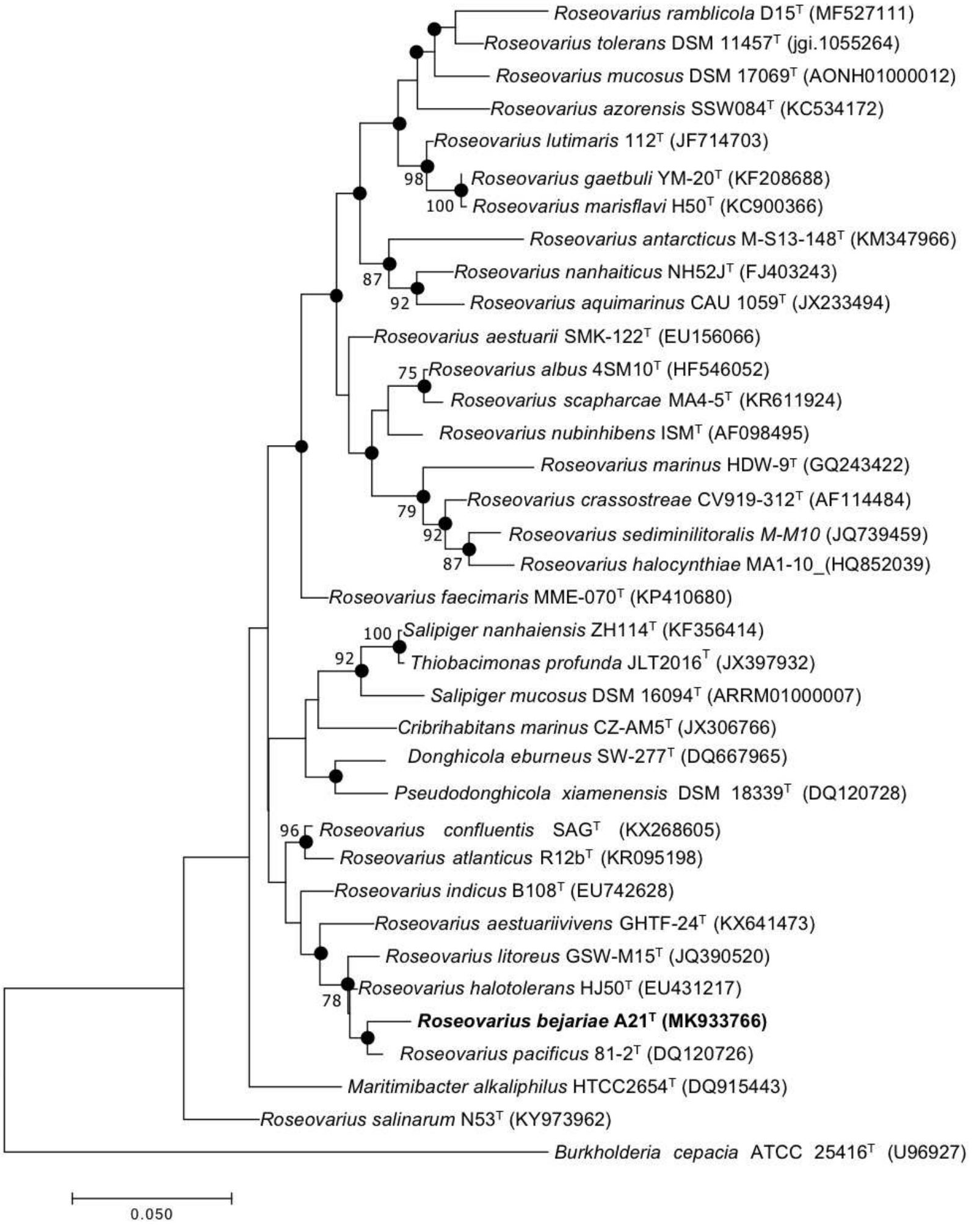
DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

ANI: Average Nucleotide Identity

dDDH: digital DNA-DNA hybridization

Figure - Phylogenetic tree in EPS format

Click here to access/download;Figure - Phylogenetic tree in EPS format;Figure 1.eps



Roseovarius bejariae sp. nov. - a moderately halophilic bacterium isolated from a hypersaline steep-sided river bed.

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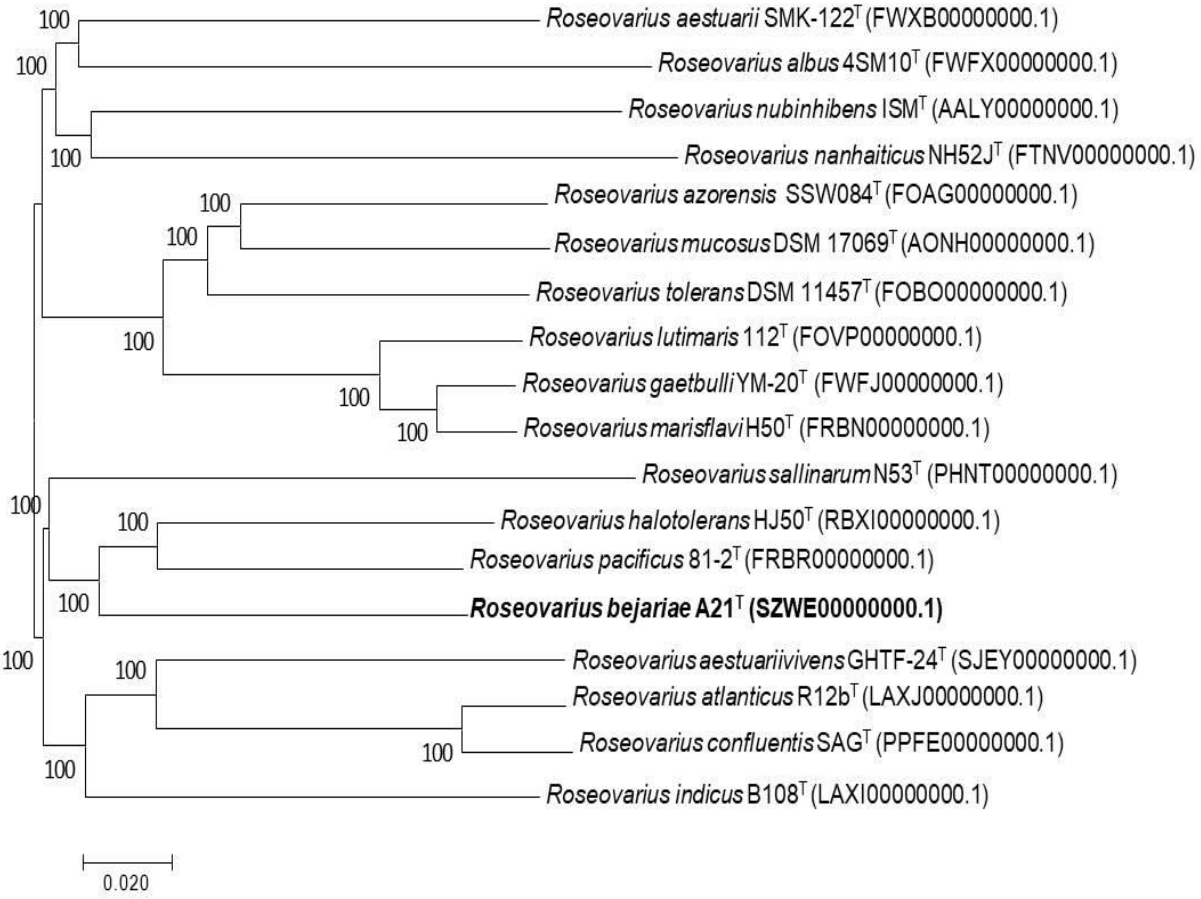
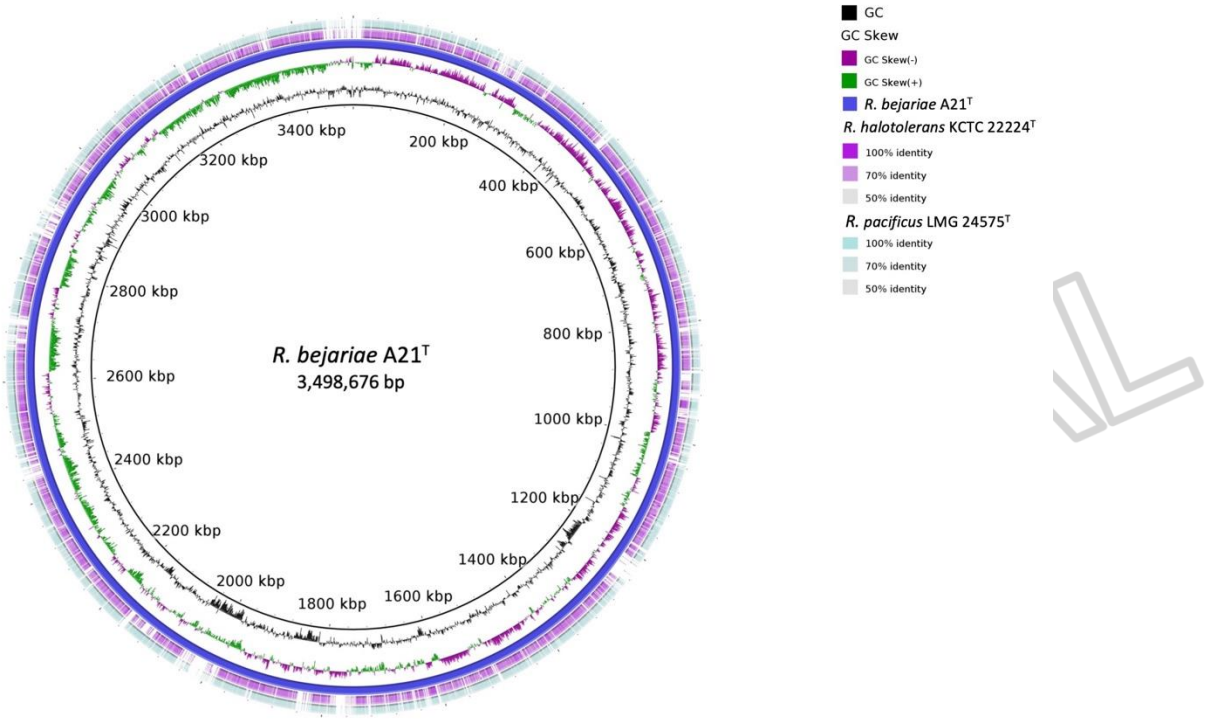
Supplementary Table 1. Assigned COG Categories of predicted proteins of A21^T strain.

Category	Name	Count	Proportion
B	Chromatin Structure and dynamics	2	0.07
C	Energy production and conversion	226	7.72
D	Cell cycle control, cell division, chromosome partitioning	26	0.89
E	Amino acid transport and metabolism	309	10.55
F	Nucleotide transport and metabolism	60	2.05
G	Carbohydrate transport and metabolism	101	3.45
H	Coenzyme transport and metabolism	122	4.17
I	Lipid transport and metabolism	105	3.58
J	Translation, ribosomal structure and biogenesis	160	5.46
K	Transcription	184	6.28
L	Replication, recombination and repair	130	4.44
M	Cell wall/membrane/envelope biogenesis	175	5.97
N	Cell motility	31	3.34
O	Posttranslational modification, protein turnover, chaperones	120	4.10
P	Inorganic ion transport and metabolism	184	6.28
Q	Secondary metabolites biosynthesis, transport and catabolism	67	2.29
S	Function unknown	746	25.47
T	Signal transduction mechanisms	109	3.72
U	Intracellular trafficking, secretion, and vesicular transport	37	1.26
V	Defense mechanisms	35	1.19

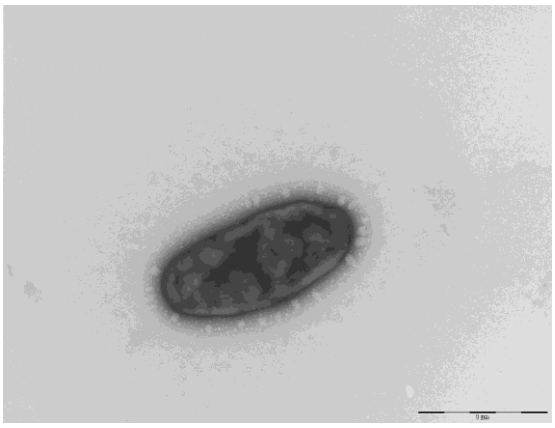
Supplementary Fig. S1. Phylogenomic tree of *Roseovarius bejariae* A21^{T} and the rest of *Roseovarius* species. The whole genomes were analyzed using BPGA and afterwards, the tree was constructed using the Neighbor-Joining method, with bootstrap analysis (1000 replications).



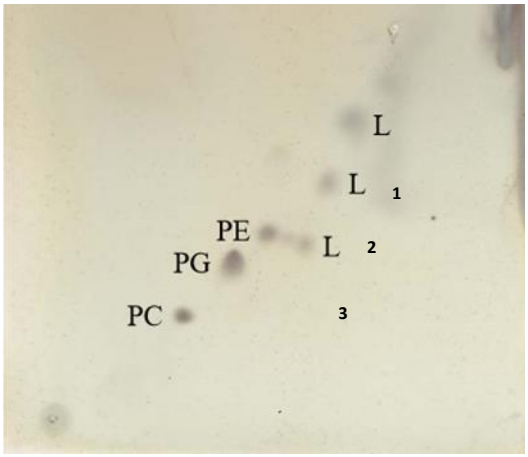
Supplementary Fig. S2. Comparison of the chromosomes of *Roseovarius bejariae* A21^{T} and the closest species: *R. halotolerans* KCTC 22224^{T} and *R. pacificus* LMG 24575^{T}. The outermost ring represents the genome of *R. pacificus* LMG 24575^{T}, and the second outermost ring belongs to *R. halotolerans* KCTC 22224^{T}. The blue ring is the whole genome of *R. bejariae* A21^{T}, that was used as reference.



Supplementary Fig. S3. Electron micrograph of negatively stained preparation of a cell of strain A21^T. Cells were grown on marine broth at 30ºC for 24 h with shaking at 150 rpm and stained with 1 % (w/v) uranyl acetate (Bar, 1 μm).



Supplementary Fig. S4. Polar lipid profile of A21^T after two dimensional TLC and detection with molybdatophosphoric acid. L, unidentified polar lipids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol and PC, phosphatidylcholine. Polar lipids are separated by two dimensional silica gel thin layer chromatography (Macherey-Nagel Art. No. 818 135). Total lipid material was detected using molybdatophosphoric acid and specific functional groups detected using spray reagents specific for defined functional groups [42].



Supplementary Fig. S5. Agarose gel (1% w/v) showing PCR products of *pufLM* genes amplified from total DNA of A21^{T} (lane 5), *Roseovarius pacificus* LMG 24575^{T} (lane 3) as negative control, *R. tolerans* EL-172^{T} (lane 4) as positive control. Lane 1, size marker (HyperLadder™ 1kb, Bioline); Lane 2, PCR negative control water.