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Silicimonas algicola gen. nov., sp nov., a member of the Roseobacter clade isolated from the cell surface of the marine diatom *Thalassiosira delicatula*

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Abstract :

A Gram-negative, aerobic, non-motile bacterium, designated strain KC90B(T), was isolated from the surface of a cell of the marine diatom Thalassiosira delicatula. The bacterial cells were pleomorphic and formed very small, beige colonies on marine agar. Optimal growth was obtained at 25 degrees C, at pH 6.5-7.5 and in the presence of 1.5-2.0% (w/v) NaCl. Phylogenetic analyses based on its 16S rRNA gene sequence revealed that strain KC90B(T) belonged to the Roseobacter clade and formed a monophyletic cluster with the sequences of Boseongicola aestuarii, Profundibacterium mesophilum, Hwanghaeicola aestuarii, Maribius pelagius and M. salinus, showing 91.4-95.7% sequence similarities. Ubiquinone Q-10 was the predominant lipoquinone but a significant amount of ubiquinone Q-9 was also detected. The major cellular fatty acids were C-18:1 omega 7c, 11-methyl C-18:(1)omega 7c and C-18:0. Strain KC90B(T) also contained specific fatty acids (C-17:0, anteiso-C-15:0 and anteiso-C-17:0) that were not detected in its closest described relatives. The major polar lipids of strain KC90B(T) comprised phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol and an unidentified aminolipid. The DNA G+C content of strain KC90B(T) was 65.2 mol%. The phylogenetic analysis of strain KC90B(T), together with the differential phenotypic and chemotaxonomic properties demonstrate that strain KC90B(T) is distinct from type strains of B. aestuarii, P. mesophilum, H. aestuarii, M. pelagius and M. salinus. Based on the data presented in this study, strain KC90B(T) represents a novel genus and species within the family Rhodobacteraceae, for which the name Silicimonas algicola gen. nov., sp. nov. is proposed. The type strain is KC90B(T) (=DSM 103371(T)=RCC 4681(T)).

Keywords : Silicimonas algicola, Roseobacter clade, algal-bacterial interactions, Thalassiosira.

Alphaproteobacteria are the most abundant heterotrophic bacteria found in marine pelagic environments (Zinger *et al.*, 2011) with a high contribution of the *Roseobacter* clade (family *Rhodobacteraceae*)(Buchan *et al.*, 2005; Luo & Moran, 2014). Members of the *Roseobacter* clade are often dominant in natural assemblages with marine algae and have been shown to increase in abundance during phytoplankton blooms (Amin *et al.*, 2012; Buchan *et al.*, 2014; Gonzalez *et al.*, 2000; Mayali *et al.*, 2008; Zubkov *et al.*, 2001). They also are often found in laboratory cultures of marine phytoplankton (Alavi *et al.*, 2001; Amin *et al.*, 2012; Grossart *et al.*, 2005; Jasti *et al.*, 2005) and both mutualistic (Geng & Belas, 2010; Wagner-Döbler *et al.*, 2010) and pathogenic (Boettcher *et al.*, 2005; Seyedsayamdost *et al.*, 2011) lifestyles have been suggested. To date, numerous *Roseobacter* clade genomes have been sequenced, revealing versatile metabolic capabilities that partly explain the success of the clade in marine environments. They gain energy from the oxidation of a multitude of organic compounds, and some members are also capable of phototrophy. Light utilization involving bacteriochlorophyll *a* (BChl *a*) by aerobic anoxygenic phototrophs (Moran *et al.*, 2004; Swingley *et al.*, 2007; Wagner-Döbler *et al.*, 2010) and based on rhodopsins (Newton *et al.*, 2010; Voget *et al.*, 2015) is found in phylogenetically diverse strains. Recently, Pujalte *et al.* (2014) divided the *Roseobacter* clade into 68 genera that correspond to 164 species but new genera and species have been described afterwards, including the genera *Boseongicola* (Park *et al.*, 2014), *Pseudoseohaeicola* (Park *et al.*, 2015), and *Xuhuaishuia* (Wang *et al.*, 2016). However, many other *Roseobacter* lineages do not have cultivated members.

In a study investigating the specificity of bacteria attached to marine diatom cells in laboratory cultures, we isolated bacteria attached to the cell walls of *Thalassiosira delicatula* RCC 2560 (Roscoff Culture Collection, France). This microalgal culture isolated from surface water at the coastal long-term monitoring station SOMLIT-Astan site (48°45’ N, 3°57’ W, north off Roscoff, Western English Channel) is maintained in the RCC since its isolation in January 2011. To isolate attached bacteria, single diatom cells were isolated under sterile conditions in a laminar flow hood. Algal cells were first gently separated by gravity using a 47 mm diameter, 11 µm pore-size nylon filter (Millipore) and washed three times with 50 mL of autoclaved seawater in order to lower the number of free-living bacteria in the algal culture. Single diatom cells were then picked with a sterile glass capillary micropipette and washed 3-4 times with filter-sterilized seawater. Controls were performed for each diatom cell isolated by checking

the absence of bacteria in the last drop of seawater used in the washing series. For cultivation of diatom epibionts, single isolated algal cells and controls were directly transferred in 48-well plates containing low-nutrient heterotrophic medium (LNHM) (Rappé *et al.*, 2002) prepared by dissolving 35 $g.l^{-1}$ of commercial sea salts (Red Sea Europe) instead of using natural seawater. Bacterial cultures were incubated at 19°C for 3 to 4 weeks and growth was analysed by flow cytometry using a BD Accuri C6 cytometer (BD Biosciences). Cultures that contained bacteria were streaked on LNHM agar for purification at least two times. Strain KC90B^T was one of the resulting isolates. Strain KC90B^T was further cultivated routinely in modified Marine Agar (1:10; 0.5 g peptone, 0.1 g yeast extract, 35 g sea salts dissolved in 1 l of Milli-Q water and 15 g agar) and in modified Marine Broth (MB) (1:2; 2.5 g peptone, 0.5 g yeast extract, 35 g sea salts dissolved in 1 l of Milli-Q water). The bacterial culture was then stored at -80 °C in the presence of 7.5% (v/v) DMSO.

Phenotypic characteristics of strain KC90B^T including growth, physiological and biochemical properties were tested as follows. Cell morphology and motility were examined using phase-contrast light microscopy (BX51; Olympus) and transmission electron microscopy (TEM) (JEM-1400, JEOL). TEM was performed after negative staining of cells with 2% uranyl acetate on Formvar-carbon-coated 400 mesh copper grids. Gram staining was performed according to (Smibert & Krieg, 1994). Growth at various temperatures (4-45 °C) and pH (4.5-10.5) were determined in MB (1:2). Media used to determine pH range for growth were adjusted using the following buffers: CH_3COONa 2M/acetic acid 2M for pH 4.5 to 5.5, Na_2HPO_4 2M/NaH_2PO_4 2M for pH 6 to 8.5 and Na_2CO_3 1M/$NaHCO_3$ 1M for pH 9 to 10.5. The media were sterilized by filtration using 0.1µm pore size PES membrane filter units (Nalgene™ Rapid-Flow™). The requirement and tolerance to NaCl was tested in MB (1:2) using increasing concentrations of NaCl from 0 to 3 % (w/v) in increments of 0.5% and from 3 to 8 % in increments of 1%. Bacterial growth was assessed by flow cytometry. For flow cytometry, 100 µl cultures were fixed with glutaraldehyde (0.25%, final concentration) and stained with Sybr Green (Life Technologies) (Marie *et al*. 1997). Susceptibility to antibiotics was evaluated by spreading a bacterial suspension (200 µl) with a turbidity of 1-2 McFarland on MA (1:2) plates using susceptibility disks (bioMérieux) containing ampicillin (10 µg), chloramphenicol (30 µg), penicillin G (10 IU), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), rifampicin (5 µg), erythromycin (15 µg) and neomycin (30 µg). Enzyme activities were determined after incubation at optimal growth temperature for 4 days, by using the API

ZYM system (bioMérieux). Assimilation tests were performed using the API 20 NE and API 50 CH systems incubated at optimal growth temperature for 15 days. All API test kits were used following the manufacturer's instructions except that the inoculating medium consisted of sterile Red Sea salts (35 ppt salinity) supplemented with mix of trace metals and vitamins solutions used in Carini *et al*. (2013). Catalase and oxidase activities were determined as described by Smibert & Krieg (1994).

Genomic DNA was extracted using lysis and neutralization buffers prepared as described in Humily *et al.* (2014). Briefly, after addition of 0.5 µl of lysis buffer, the mixture was incubated at 4°C for 10 min in a thermocycler. The lysate was further incubated at 95°C for 1 min, cooled at 4°C before adding 0.5 µL of neutralization buffer, and kept 3 min on ice until amplification by PCR. The 16S rRNA gene of KC90B^T was amplified using the primers 8F and 1492R (Turner *et al.*, 1999). The reaction mixture (12.5µL) contained 1µL of cell lysate, 0.1 mM of each deoxynucleoside triphosphate, 1X Green GoTaq Flexi Buffer, 2.0 mM $MgCl_2$, 0.2 µM of each primer, and 0.75 U of GoTaq G2 Flexi DNA polymerase (Promega). Conditions for PCR were as follows: 95°C for 10 min followed by 35 cycles (95°C for 30 s, 55°C for 1 min and 72°C for 1 min), and a final extension step for 10 min at 72°C. Sequencing was carried out using an Applied Biosystem 3100 automated DNA sequencer (Biogenouest platform, Station Biologique de Roscoff). The resulting 16S rRNA gene sequence (1395 nt) was compared by BLASTn with sequences available in GenBank. Phylogenetic analysis was performed using the neighbor joining, maximum parsimony and maximum likelihood inference approaches implemented in MEGA6 software (Tamura *et al.*, 2013). To amplify partial sequences of the *pufM* gene, coding for of the M subunit of the photosynthetic reaction centre, the PufMF forward (5'-TACGGSAACCTGTWCTAC-3', Béjà *et al.*, 2002) and Puf-WAW reverse primers (5'-AYNGCRAACCACCANGCCCA-3', Yutin *et al.*, 2005) were used according to Lehours *et al*. (2010). For proteorhodopsin detection, the set of degenerated primers PR-1aF (5'-GATCGAGCGNTAYRTHGAYTGG-3') and PR-1aR (5'-GATCGAGCRTADATNGCCCANCC-3') was employed using conditions described by Campbell *et al.* (2008).

For genome analyses, genomic DNA was isolated from 500 mg harvested cells grown in MB (1:2) at 20°C after 15 days. The genome size and DNA G+C content were directly calculated from the complete genome sequence of the strain KC90B^T. Complete genome sequencing was carried out using the PacBio *RSII* System (Pacific Biosciences, Menlo Park, CA) at the Leibniz-Institut DSMZ. This calculation method differs from

conventional indirect methods used for the five reference strains [HPLC according to Tamaoka & Komagata (1984) or Mesbah *et al.* (1989)], but calculation of G+C content directly from genome is more accurate (Meier-Kolthoff *et al.*, 2014) and differences between two methods are between 1.2 and 2% (Mesbah *et al.*, 2011).

Cells (0.2 to 0.5 µm wide and 0.2 to 17 µm long) are Gram-negative, aerobic, non-flagellated and pleomorphic (few coccoids, some ovoids and mainly rod-shaped cells of various lengths) (Supplementary Fig. 1). Colonies on MA are circular, slightly convex, glistening, beige and 0.3–1 mm in diameter after incubation for 14 days at 25°C. The distinctive morphological, cultural, physiological and biochemical characteristics of strain KC90B^T are given in the genus and species descriptions (see below) and in Table 1.

Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain KC90B^T formed a distinct lineage within the *Roseobacter* clade in the family *Rhodobacteraceae* of the *Alphaproteobacteria* (Figure 1), the nearest described relatives being *Boseongicola aestuarii* (95.7%), *Maribius pelagius* (94.0%), *M. salinus* (94.0%), *Profundibacterium mesophilum* (93.5%), and *Hwanghaeicola aestuarii* (91.4%). The lineage did not associate significantly with any of the currently described genera in the family. Interestingly, strain KC90B^T shared a higher sequence similarity (96.6%) with undescribed strain DG981 isolated from a culture of the toxic dinoflagellate *Gymnodinium catenatum* GCTRA14, originating from Spring Bay in Tasmania (Green *et al.*, 2004). The branching orders and phylogenetic relationships between strain KC90B^T and DG981-*Boseongicola-Profundibacterium-Maribius-Hwanghaeicola* were well conserved in the phylogenetic trees reconstructed using neighbor-joining, maximum-parsimony and maximum-likelihood algorithms.

The robustness of the phylogenetic relationships and the low sequence similarities between the strains and the other genera demonstrate that the novel isolate represents a new genus in the family *Rhodobacteraceae*.

The estimated genome size, based on genome sequencing data, was approximately 4.4 Mbp. The DNA G+C content of strain KC90B^T was 65.2 mol% as computed from genome sequences. Conclusively, no genes for *pufM* and proteorhodopsin could be detected for KC90B^T using PCR (data not shown).

For fatty acid analysis, cells were grown in liquid Marine broth for 10 days at 25°C. Data taken from the literature were obtained under growth conditions comparable to those

used for strain KC90B^T (Park *et al.*, 2014). After harvesting the biomass, cells were extracted according to the standard protocol (Sasser, 1990) of the Microbial Identification System (MIDI Inc.; version 6.1). The fatty acids were identified by comparison to the TSBA40 peak-naming table database. Strain KC90B^T has straight-chain, methyl- or hydroxy-branched saturated and monounsaturated fatty acids. The major fatty acid (>10% of the total fatty acids) detected in strain KC90B^T was $C_{18:1}\omega7c$ (60.0%). The fatty acid profile of the reference strain BS-W15^T showed the same prevalence of the fatty acid $C_{18:1}\omega7c$ (Park *et al.*, 2014). However, the fatty acid profile of KC90B^T is distinguishable from BS-W15 due to differences in fatty acid composition (Table 2). KC90B^T contains 2 anteiso fatty acids (*anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$) while BS-W15 ^{T} does not have any. In addition, the fatty acid *cyclo*$C_{19:0}\omega8c$ (0.9%) and the unknown fatty acid 11.799 (2.8%) were detected in KC90B^T but not in BS-W15^T.

Isoprenoid quinones were extracted from dried biomass with chloroform/methanol (2:1, v/v; Collins & Jones, 1981) and analysed via HPLC (Tindall, 1990). A large amount of ubiquinone Q-10 was detected (81.5%) which is typical of the *Alphaproteobacteria* class. In addition, a significant amount of ubiquinone Q-9 (18.4%) was detected. This profile differs from the one of BS-W15^T where ubiquinone Q-10 (predominant), Q-8 (16.0 %) and Q-9 (2.0 %) were detected (Park *et al.*, 2014).

The polar lipid composition of strain KC90B^T was analysed by two-dimensional TLC (modified after Bligh & Dyer, 1959, Tindall *et al.*, 2007). The major polar lipids detected were phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol and an unidentified aminolipid (Figure 2). In addition, minor amounts of three unidentified glycolipids, three unidentified phospholipids, one unidentified aminolipid and one unidentified lipid were detected. Compared to closely related genera, the polar lipid profile of strain KC90B^T is quite distinguishable. The strain *Boseongicola aestuarii* BSW15^T, *Profundibacterium mesophilum* JCM 17812^T, *Hwanghaeicola aestuarii* KACC 13705^T, *Maribius pelagius* KCCM 42336^T and *Maribius salinus* KCCM 42113^T do not present any glycolipids except for *H. aestuarii* KACC 13705^T. Except for *M. pelagius* KCCM 42336^T and *M. salinus* KCCM 42113^T, they all present low amounts of aminolipids (Park *et al.*, 2014). The polar lipid profile of strain KC90B^T is also distinguishable from other phylogenetically related genera of the *Roseobacter* clade such as *Marivita*, *Roseovarius* and *Litoreibacter* because of the absence of phosphatidylethanolamine as a major component (Hwang *et al.*, 2009; Kim *et al.*, 2012; Park & Yoon, 2013).

Strain KC90B^T was differentiated from the type strains *B. aestuarii, P. mesophilum*, *H. aestuarii*, *M. pelagius* and *M. salinus* by differences in its phenotypic characteristics, including cell morphology, motility, optimal temperature, salinity and pH for growth, assimilation of some substrates, susceptibility to antibiotics and some enzymatic activities. The phylogenetic and chemotaxonomic analyses and the different tested properties conclusively demonstrated that strain KC90B^T represents a novel genus and species in the *Roseobacter* clade (family *Rhodobacteraceae*, order *Rhodobacterales*), for which the name *Silicimonas algicola* gen. nov., sp. nov. is proposed.

Description of *Silicimonas* gen. nov.

Silicimonas [Si.li.ci.mo'nas L. n. *silex*, silica; L. fem. n *monas*, a monad, a unit; N.L. fem. n. *Silicimonas*, a monad isolated from silica]

Cells are Gram-negative, aerobic, non-flagellated and pleomorphic (few coccoids, some ovoids and mainly rod-shaped cells of various lengths). Catalase and oxidase positive. The major fatty acid is $C_{18:1}$ ω7c. The predominant ubiquinone is Q-10. The major polar lipids are phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol and an unidentified aminolipid. The genus is a member of the class *Alphaproteobacteria*, order *Rhodobacterales*, family *Rhodobacteraceae*. The type, and only species is *Silicimonas algicola*.

Description of *Silicimonas algicola* sp. nov.

Silicimonas algicola (al.gi'co.la. L. fem. n. *alga* alga or seaweed; L. suff. -*cola* from L. n. *incola* an inhabitant or dweller; N. L. fem. n. *algicola* alga dweller)

Cells are 0.2 to 0.5 µm wide and 0.2 to 17 µm long. Colonies on MA are circular, slightly convex, glistening, beige and 0.3–1 mm in diameter after incubation for 14 days at 25°C. Growth occurs at 10-40 °C (optimum 25°C), pH 6 to 9 (optimum 6.5-7.5), and 0.5–4% (w/v) NaCl (optimum 1.5-2%). No growth was obtained at 4°C or 45°C, at pH 5.5 and 9.5, and at NaCl concentrations of 0 and 4.5% (w/v). Nitrate reduction is negative. D-mannose, arbutin, esculine ferric citrate and potassium 2-ketoglutanate are utilized, but not glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-ßD-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, N-acetylglucosamine, amygdalin,

salicin, D-cellobiose, D-maltose, D-lactose (bovine origin), D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 5-ketogluconate. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase activities are present, but lipase (C14) is weakly present and cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent.

The major fatty acids are $C_{18:1}$ ω7c, 11-methyl $C_{18:1}$ ω7c and $C_{18:0}$.

The predominant ubiquinone is Q-10.

The major polar lipids are phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol and an unidentified aminolipid.

The DNA G+C content of the type strain is 65.2 mol% by whole genome sequencing.

The type strain, KC90B^T (=DSM 103371^T=RCC 4681^T), was isolated from the silica cell wall of *Thalassiosira delicatula* RCC 2565, a marine diatom originating from Roscoff offshore seawater in the western English Channel.

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Figure captions

Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain KC90B^T and representatives of some related taxa. Only bootstrap values (expressed as percentages of 1000 replications) of > 40% are shown. Filled circles indicate that the corresponding nodes were also recovered using the maximum-likelihood and maximum-parsimony algorithms, while open circles indicate that the corresponding nodes were also recovered using the maximum-likelihood method. *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Figure 2. Thin layer chromatograms of polar lipids of strain KC90B^T. GL1-GL3, unidentified glycolipids ; PL1-PL3, unidentified phospholipids; PC, phosphatidylcholine ; PG, phosphatidylglycerol ; AL, unidentified aminolipid ; DPG, diphosphatidylglycerol ; L, unidentified lipid.

Supplementary Figure S1. Transmission electron micrograph showing the pleomorphic forms [coccoid (a), ovoid (b), and rod-shaped (c)] of negatively stained cells of strain KC90B^T after growth for 10 days at 25°C in MB (1:2). Bar, 5 μm.

Table 1. Differential phenotypic characteristics of strain KC90B^T and the type strains of phylogenetically related species. Strains: 1. KC90B^T ;2. *Boseongicola aestuarii* BS-W15^T;3. *Profundibacterium mesophilum* JCM 17872^T;4. *Hwanghaeicola aestuarii* KACC 13705^T;5. *Maribius pelagius* KCCM 42336^T; 6. *Maribius salinus* KCCM 42113^T. Data obtained from this study and from Choi *et al*. (2007), Kim *et al*. (2010), Lai *et al*. (2013) and Park *et al*. (2014). +, positive reaction; -, negative reaction; w, weakly positive reaction; ND, not determined. All strains are positive for the following enzymatic activities: activity of esterase lipase (C8), leucine arylamidase, oxidase and catalase. All strains are negative for the following activities: acid production from D-melibiose, activity of trypsine, α-galactosidase, α-mannosidase, α-fucosidase, nitrate reduction.

Characteristics	1	2	3	4	5	6
	Culture of *Thalassiosira delicatula*	Tidal flat sediment at Boseong (South Korea)	Deep-sea sediment (Red Sea)	Tidal flat (Yellow Sea)	Surface water (Sargasso Sea)	Hypersaline water of a solar saltern (Korea)
Cell morphology	Pleomorphic	Pleomorphic	Coccoid	Coccoid	Rod-shaped	Rod-shaped
Motility	-	-	-	+	-	-
Optimal growth temperature (°C)	25	25	20–25	25–30	30–35	30–35
Growth temperature range (°C)	10-40	10-30	15-25	15-35	10-40	10-35
Optimal growth pH	6.5-7.5	7-8	7-8	6.5-7.5	ND	ND
Growth pH range	6-9	6.5-9.5	6-8.5	6-8	6-9	7-8
Optimal growth NaCl (%)	1.5-2	2	2-6	2-3	ND	ND
Growth NaCl range (%)	0.5-4	0.5-5	0.4-24	1.5-6	2-15	1-10
Colony size (mm)	0.3-1	0.4-0.8	0.1-0.3	ND	ND	ND
Colony color	beige	Yellowish-white	transparent	pale pink	beige	beige
Growth time on MA (days)	15	10	10	3-5	15-30	15-30
Assimilation of:						
Glycerol	-	ND	+	ND	+	-
L-arabinose	-	-	-	-	+	w
D-ribose	-	-	-	+	+	-
D-xylose	-	-	+	+	+	+
D-galactose	-	-	+	-	-	-
D-glucose	-	+	+	-	+	-
D-fructose	-	+	-	w	+	+
D-mannose	+	+	-	-	-	-
L-rhamnose	-	+	-	+	-	-
Inositol	-	-	-	-	-	+
D-mannitol	-	-	-	-	-	+
D-sorbitol	-	-	-	-	-	+
N-acetylglucosamine	-	ND	ND	ND	+	+
D-cellobiose	-	+	-	w	-	+
D-maltose	-	+	-	-	-	-
D-lactose (bovine origin)	-	+	-	-	-	-
D-trehalose	-	+	-	-	-	-
D-raffinose	-	+	-	-	-	+
L-tryptophane	-	ND	-	+	-	-
L-arginine	-	ND	+	ND	+	+
Urea	-	-	-	+	+	+
Gelatin	-	-	-	+	-	-
D-glucose	-	+	-	-	-	-
L-arabinose	-	-	-	-	+	w
D-mannose	-	+	-	-	-	-
D-mannitol	-	-	-	+	-	-
N-acetylglucosamine	-	ND	ND	-	+	+
D-maltose	-	+	-	-	-	-
Trisodium citrate	-	ND	+	-	+	+
Susceptibility to:						
Ampicillin	+	-	+	-	+	+
Chloramphenicol	+	-	+	+	+	+
Penicillin G	+	-	+	+	+	+
Gentamicin	w	-	+	-	+	+
Kanamycin	+	-	-	-	+	+
Streptomycin	+	-	+	-	+	-
Tetracycline	+	+	-	-	+	+
Nalidixic acid	-	ND	-	ND	-	-
Erythromycin	+	ND	ND	ND	+	+
Neomycin	+	+	+	+	+	+
Enzyme activity (API ZYM):						
Alkaline phosphatase	+	+	+	+	-	-
Esterase (C4)	+	+	-	+	+	+
Lipase (C14)	w	-	-	w	-	-
Valine arylamidase	+	-	+	w	-	-
Cystine arylamidase	-	-	-	w	-	-
α-chymotrypsin	-	-	-	w	-	-
Acid phosphatase	+	+	+	w	-	-
Naphthol-AS-BI-phosphohdrolase	+	-	+	w	-	-
β-galactosidase	+	-	+	-	**+**	**+**
β-glucuronidase	-	-	-	**w**	-	-
α-glucosidase	+	-	-	**-**	-	-
β-glucosidase	+	-	-	-	-	-
N-acetyl-β-glucosaminidase	-	-	+	-	-	-
DNA G+C content (mol%)	65.2	58.7	64..0	61.0	66.7	70.0

525 **Table 2.** Cellular fatty acid composition (%) of strain KC90B^T and its closest validly named
526 relative BS-W15^T (data from Park *et al.*, 2014).
527
528
529

Fatty acid	KC90B^T	BS-W15^T
Straight- chain		
$C_{16:0}$	3.6	1.8
$C_{17:0}$	0.5	-
$C_{18:0}$	5.9	5.5
Unsaturated		
$C_{18:1}$ *ω7c*	60.0	73.1
$C_{18:1}$ *ω9c*	1.8	1.7
$C_{20:1}$ *ω7c*	-	0.9
Hydroxy		
$C_{10:0}$ 3-OH	2.5	2.2
$C_{12:0}$ 3-OH	0.7	<0.5
Methyl-branched		
anteiso-$C_{15:0}$	1.3	-
anteiso-$C_{17:0}$	0.7	-
11-methyl $C_{18:1}$ *ω7c*	8.4	12.9
cyclo* $C_{19:0}$ *ω8c	0.9	-
Unknown 11.799	2.8	-
Summed features		
3 ($C_{16:1}$ *ω7c* / $C_{16:1}$ *ω6c)*	0.6	0.8
7($C_{19:1}$ *ω6c* / unknown 18.846 / *cyclo*-$C_{19:1}$ *ω10c*)	0.6	0.7

Figure 1

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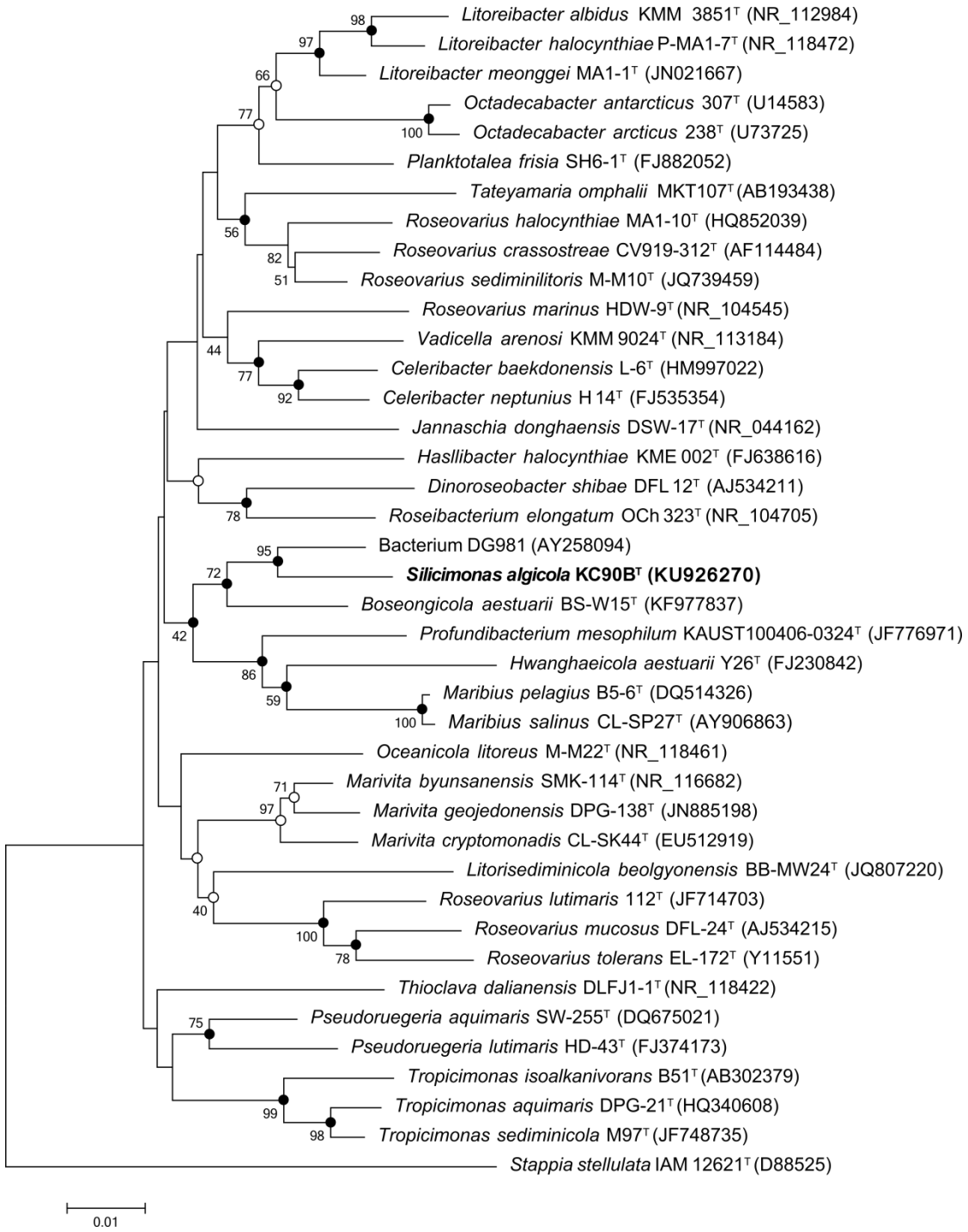


Figure 1

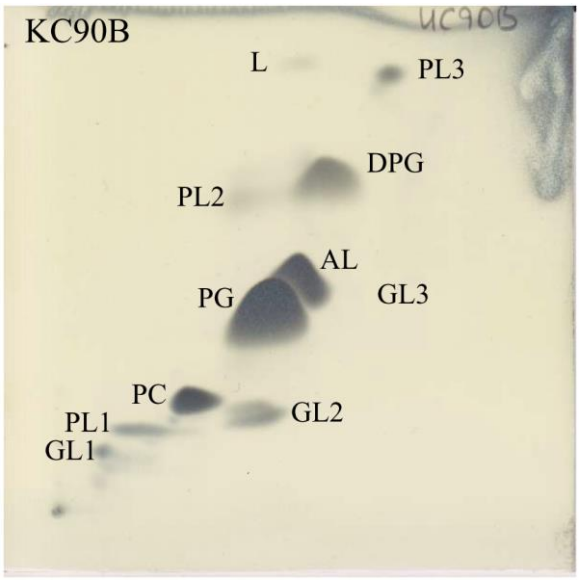
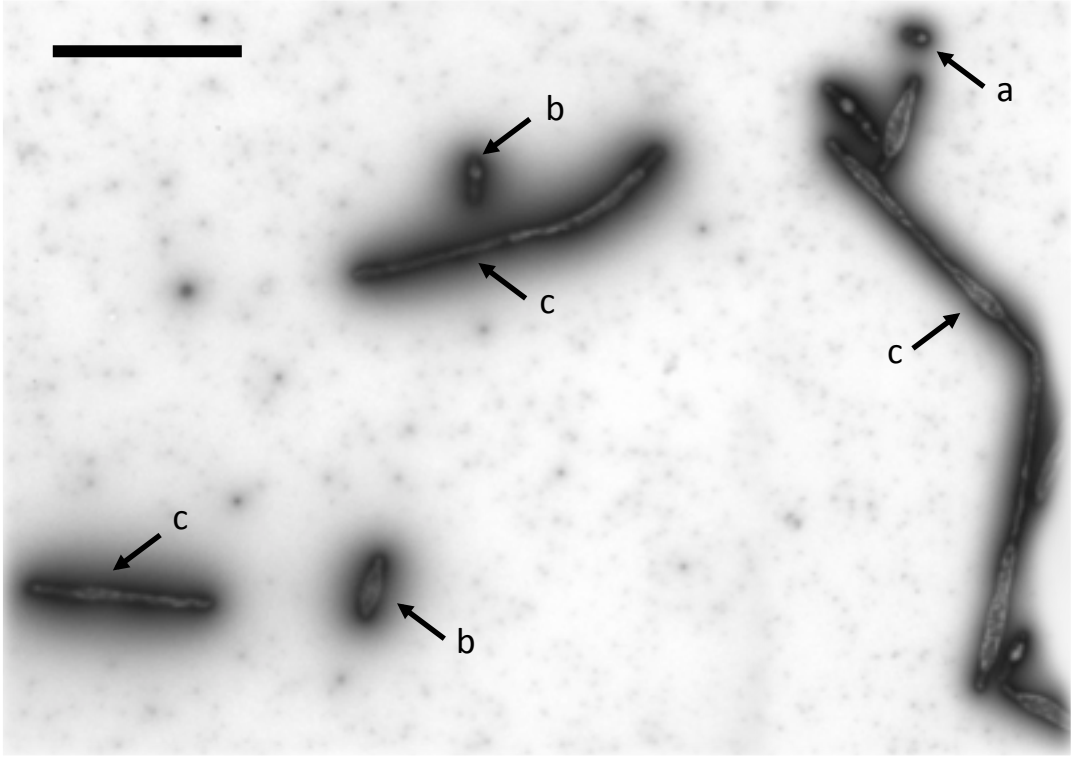


Figure 2



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