Cesiribacter roseus sp. nov., a pink-pigmented bacterium isolated from desert sand

Ming Liu,¹ Huan Qi,¹ Xuesong Luo,² Jun Dai,¹ Fang Peng¹ and Chengxiang Fang¹

Correspondence

Fang Peng

fangpeng2@yahoo.com.cn

Chengxiang Fang

cxfang@whu.edu.cn

¹College of Life Sciences, Wuhan University, Wuhan 430072, PR China

²School of Environmental Studies, China University of Geosciences, Wuhan 430074, PR China

A pink-pigmented, Gram-negative, rod-shaped, motile, strictly aerobic bacterium, designated strain 311^T, was isolated from desert sand in Xinjiang, China. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain 311^T was related closely to *Cesiribacter andamanensis* AMV16^T (94.6% similarity). The DNA G+C content of strain 311^T was 47.1 mol% and the major respiratory quinone was menaquinone 7 (MK-7). The main cellular fatty acids were C_{16:1}ω5c (29.9%), iso-C_{15:0} (21.9%), iso-C_{17:0} 3-OH (13.3%) and summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B; 13.0%). Based on phenotypic and chemotaxonomic data and phylogenetic analysis, strain 311^T is considered to represent a novel species of the genus *Cesiribacter*, for which the name *Cesiribacter roseus* sp. nov. is proposed. The type strain is 311^T (=CCTCC AB 207142^T =KACC 15456^T).

The genus *Cesiribacter* was proposed by Srinivas *et al.* (2011) based on the description of a Gram-negative, non-motile, aerobic bacterium with high DNA G+C content. The genus was related phylogenetically to the genus *Marivirga* of the family *Flammeovirgaceae*. At the time of writing, the genus comprises one recognized species, *Cesiribacter andamanensis*.

The family *Flammeovirgaceae* includes several genera, including *Aureibacter*, *Cesiribacter* (Srinivas *et al.*, 2011), *Flammeovirga*, *Flexithrix*, *Limibacter*, *Marinoscillum* (Seo *et al.*, 2009), *Marivirga* (Nedashkovskaya *et al.*, 2010), *Pere-xilibacter*, *Persicobacter*, *Roseivirga* (Nedashkovskaya *et al.*, 2005a; Lau *et al.*, 2006), *Rapidithrix*, *Sediminitomix* and *Thermonema*, and was described by Yoon *et al.* (2011). During investigation of the microbial diversity of desiccated environments of the Taklimakan desert of Xinjiang, China, numerous bacteria were isolated, including a pink-pigmented bacterial strain, designated 311^T, isolated from a sand sample. For isolation, 1 g aliquots of sand were serially diluted in saline water (0.85%, w/v) and plated onto tenfold-diluted trypticase soy agar (0.1 × TSA; Difco). After incubation at 30 °C for 20 days, single colonies on the plates were purified. Strain 311^T was stored by lyophilization.

Genomic DNA extraction and amplification of the 16S rRNA gene of strain 311^T were carried out as described by Rainey *et al.* (1996). PCR products were sequenced by Invitrogen Biotechnology. Similarity searches with the

derived sequences were performed via the EzTaxon server (Chun *et al.*, 2007). Phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data via CLUSTAL X (Thompson *et al.*, 1997). Distances were obtained by using options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic trees created with the neighbour-joining method was evaluated by using bootstrap resampling (Felsenstein, 1985) with 1000 replications (Fig. 1).

Cell morphology was examined by phase-contrast microscopy (Olympus) and by transmission electron microscopy. A number of key characteristics were also tested by using standard procedures (Gerhardt *et al.*, 1994): KOH string test (Gram stain) and oxidase and catalase (3% H₂O₂). Nitrate reduction and hydrolysis of aesculin, casein, starch and gelatin were tested according to Gerhardt *et al.* (1994). Flexirubin-type pigments were detected by using the simple KOH test detailed in the minimal standards for describing novel taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). Growth at 4, 12, 18, 25, 30, 37, 42 and 45 °C and with 0, 1.0, 2.0, 3.0 and 4.0% NaCl was tested in tenfold-diluted trypticase soy broth (0.1 × TSB); growth at pH 6–11 (intervals of 1 pH unit) was determined in 0.1 × TSB with 1% NaCl. Anaerobic growth was assessed on 0.1 × TSA (both with and without KNO₃) incubated in air-tight jars containing an AnaeroPack (Oxoid). Antibiotic resistance was determined with the disc diffusion method (Buczolits *et al.*, 2002) on 0.1 × TSA incubated for up to 3 days at 30 °C.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain 311^T is HM775387.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between strain 311^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) >70% are shown at nodes. *Escherichia coli* ATCC 11775^T was used as an outgroup (not shown). Bar, 0.02 changes per nucleotide position.

Strain 311^T was also characterized by using the whole test spectrum of the API ZYM, API 20NE and API 20E identification systems (bioMérieux) according to the manufacturer's instructions.

To determine its whole-cell fatty acid profile, strain 311^T was grown on 0.1 × TSA (Difco) with 1% NaCl at 30 °C for 4 days, and analysis of the fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (MIDI). Respiratory quinones were analysed according to the protocol of Xie & Yokota (2003). DNA was extracted from cells grown in 0.1 × TSB by using a modification of the protocol of Wilson (1987). The HPLC method of Mesbah *et al.* (1989) was used to determine the G+C content of the extracted DNA.

16S rRNA gene sequence comparisons indicated that strain 311^T was related phylogenetically to members of the genera *Cesiribacter* and *Marivirga* (Fig. 1). The phylogenetic tree showed that strain 311^T formed a separate cluster with *C. andamanensis* AMV16^T. Sequence comparisons also revealed that strain 311^T exhibited highest 16S rRNA gene sequence similarity (94.6%) to *C. andamanensis* AMV16^T and showed relatively low levels of sequence similarity (<90.0%) with respect to the type strains of other species.

Cells of strain 311^T were strictly aerobic, Gram-negative, flexirubin-positive, variably shaped rods with an extra-cellular matrix (Fig. 2). Colonies were pink, circular and convex with regular margins after growth on 0.1 × TSA at 30 °C for 3 days. Growth was observed at 4–37 °C, with good growth at 30 °C, at 0–3.0% NaCl (optimum 2.0%) and at pH 7–10 (optimum pH 8). Aesculin, gelatin, casein and starch were hydrolysed. Strain 311^T was catalase- and nitrate reductase-positive, but oxidase-negative. The results of API ZYM, API 20E, API 20NE and antibiotic susceptibility tests are listed in the species description.

Based on chemotaxonomic characteristics, strain 311^T could be easily differentiated from *C. andamanensis* AMV16^T and *Marivirga tractuosa* NBRC 15989^T. The major cellular fatty acids of strain 311^T were C_{16:1}ω5c (29.9%), iso-C_{15:0} (21.9%), iso-C_{17:0} 3-OH (13.3%) and summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B;

13.0%). In comparison, these components are present at low levels in *C. andamanensis* AMV16^T (Table 1). This fatty acid profile for *C. andamanensis* is highly atypical for members of the *Bacteroidetes*. Moreover, the DNA G+C content of strain 311^T was 47.1 mol% (HPLC), which is much higher than for the genus *Marivirga* (36–37 mol%) (Table 2) but similar to that for *C. andamanensis* AMV16^T.

The phenotypic, chemotaxonomic and phylogenetic data presented indicate that strain 311^T represents a novel species of the genus *Cesiribacter*, for which the name *Cesiribacter roseus* sp. nov. is proposed.

Fig. 2. Transmission electron micrographs of cells of strain 311^T grown on R2A agar at 30 °C for 60 h (a) and on 0.1 × TSA at 30 °C for 60 h (b). Bars, 700 nm (a) and 1 μm (b).

Table 1. Cellular fatty acid contents of strain 311^T and the type strains of phylogenetically related species

Strains: 1, 311^T; 2, *Cesiribacter andamanensis* AMV16^T (data from Srinivas *et al.*, 2011); 3, *Marivirga tractuosa* NBRC 15989^T [data from this study after growth on 0.1 × TSA (Difco) with 1 % NaCl at 30 °C for 4 days]; 4, *Roseirga echinomitans* KMM 6058^T (Nedashkovskaya *et al.*, 2005b); 5, *Marinoscillum furvescens* DSM 4134^T (Seo *et al.*, 2009). ND, Not detected; tr, <1 %.

Fatty acid	1	2*	3	4	5
C _{16:1} ω5c	29.9	ND	ND	ND	28.5
iso-C _{15:0}	21.9	3.4	44.7	20.2	8.5
iso-C _{17:0} 3-OH	13.3	ND	10.6	12.1	9.8
iso-C _{15:1}	4.0	ND	tr	20.2	3.2
C _{18:0}	3.7	ND	ND	ND	ND
iso-C _{17:0}	3.2	ND	tr	1.0	1.0
iso-C _{15:0} 3-OH	3.1	ND	5.8	4.1	ND
C _{16:0}	3.7	ND	1.7	ND	3.5
iso-C _{13:0} 3-OH	1.1	ND	ND	ND	ND
C _{18:1} ω9c	1.3	13.8	tr	ND	ND
C _{18:1} ω7c	1.2	ND	ND	ND	ND
iso-C _{13:0}	tr	ND	5.5	2.9	ND
iso-C _{17:1} ω9c	ND	ND	15.0	1.1	ND
Summed feature 3†	1.2	ND	5.9	1.0	22.2
Summed feature 4†	13.0	ND	ND	ND	1.0

**C. andamanensis* AMV16^T also contained C_{12:0} (3.0 %), C_{13:0} (3.8 %), C_{14:0} (3.0 %), anteiso-C_{14:0} (15.1 %), anteiso-C_{15:0} (4.6 %), C_{15:0} (28.7 %), C_{16:1}ω9c (23.9 %) and C_{18:1}ω9c (13.8 %), which are not listed in the table.

†Summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. Summed feature 4 comprised iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Table 2. Differential characteristics between strain 311^T and its phylogenetically closest relatives

Strains: 1, strain 311^T; 2, *C. andamanensis* AMV16^T (data from Srinivas *et al.*, 2011); 3, *Marivirga tractuosa* NBRC 15989^T (data from this study unless indicated). ND, No data available.

Characteristic	1	2	3
Motility	+	–	+
Oxidase	–	+	+
Maximum growth temperature (°C)	37	37	40
Nitrate reduction	+	+	–
Tolerance of 5 % NaCl	–	+	+
Enzyme activities			
Esterase	+	ND	–
Esterase lipase	+	+	–
N-Acetyl-β-glucosaminidase	+	ND	–
α-Galactosidase	+	ND	–
α-Glucosidase	+	ND	–
Trypsin	–	ND	+
DNA G + C content (mol%)	47.1	50.9	36.1*

*Data from Nedashkovskaya *et al.* (2010).

Description of *Cesiribacter roseus* sp. nov.

Cesiribacter roseus (ro'se.us. L. masc. adj. *roseus* rose-coloured, pink).

Cells are Gram-negative rods (1.5–3 × 0.4–0.5 μm) with an irregular fibril structure and are motile and strictly aerobic. Catalase-positive and oxidase-negative. Colonies are pink, circular and convex with regular margins. Flexirubin is detected. Growth occurs at 4–37 °C (optimum 30 °C), at pH 7–10 (optimum pH 8) and at NaCl concentrations of up to 3.0 % (optimum 2.0 %). Positive for hydrolysis of aesculin, casein, starch and gelatin. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, N-acetyl-β-glucosaminidase and α-glucosidase, but negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. In API 20NE test strips, positive reactions are observed for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase (weak) and negative reactions are observed for arginine dihydrolase, indole production, urease and glucose fermentation. Does not assimilate D-glucose, L-arabinose, maltose, D-mannose, D-mannitol, N-acetylglucosamine, adipic acid, capric acid, malic acid, potassium gluconate, trisodium citrate or phenylacetic acid. In API 20E tests, shows positive reactions for β-galactosidase (weak), tryptophan deaminase and gelatin hydrolysis, weakly positive reactions for oxidation of sucrose, melibiose, amygdalin and arabinose and negative reactions for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production, Voges–Proskauer reaction, urease, indole production and oxidation of glucose, mannitol, inositol, sorbitol and rhamnose. Susceptible to (per disc) erythromycin (15 μg), vancomycin (30 μg), acedomylin (30 μg) and penicillin (10 IU). The major quinone is MK-7. The DNA G + C content of the type strain is 47.1 mol%.

The type strain, 311^T (=CCTCC AB 207142^T =KACC 15456^T), was isolated from desert sand in Xinjiang, China.

Acknowledgements

This work was supported by the R & D Infrastructure and Facility Development Program from the Ministry of Science and Technology of the People's Republic of China (Grant203 no. 2005DKA21208).

References

- Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 52, 1049–1070.
- Bucolits, S., Denner, E. B., Vybiral, D., Wieser, M., Kämpfer, P. & Busse, H.-J. (2002). Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. *Int J Syst Evol Microbiol* 52, 445–456.

- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lau, S. C. K., Tsoi, M. M. Y., Li, X., Plakhotnikova, I., Dobretsov, S., Wu, M., Wong, P.-K., Pawlik, J. R. & Qian, P.-Y. (2006). Description of *Fabibacter halotolerans* gen. nov., sp. nov. and *Roseivirga spongicola* sp. nov., and reclassification of [*Marinicola*] *seohaensis* as *Roseivirga seohaensis* comb. nov. *Int J Syst Evol Microbiol* **56**, 1059–1065.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nedashkovskaya, O. I., Kim, S. B., Lee, D. H., Lysenko, A. M., Shevchenko, L. S., Frolova, G. M., Mikhailov, V. V., Lee, K. H. & Bae, K. S. (2005a). *Roseivirga ehrenbergii* gen. nov., sp. nov., a novel marine bacterium of the phylum ‘Bacteroidetes’, isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **55**, 231–234.
- Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Park, M. S., Mikhailov, V. V., Bae, K. S. & Park, H. Y. (2005b). *Roseivirga echinicomitans* sp. nov., a novel marine bacterium isolated from the sea urchin *Strongylocentrotus intermedius*, and emended description of the genus *Roseivirga*. *Int J Syst Evol Microbiol* **55**, 1797–1800.
- Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B. & Bae, K. S. (2010). Reclassification of *Flexibacter tractuosus* (Lewin 1969) Leadbetter 1974 and ‘*Microscilla sericea*’ Lewin 1969 in the genus *Marivirga* gen. nov. as *Marivirga tractuosa* comb. nov. and *Marivirga sericea* nom. rev., comb. nov. *Int J Syst Evol Microbiol* **60**, 1858–1863.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Seo, H.-S., Kwon, K. K., Yang, S.-H., Lee, H.-S., Bae, S. S., Lee, J.-H. & Kim, S.-J. (2009). *Marinoscillum* gen. nov., a member of the family ‘*Flexibacteraceae*’, with *Marinoscillum pacificum* sp. nov. from a marine sponge and *Marinoscillum furvescens* nom. rev., comb. nov. *Int J Syst Evol Microbiol* **59**, 1204–1208.
- Srinivas, T. N. R., Anil Kumar, P., Madhu, S., Sunil, B., Sharma, T. V. R. S. & Shivaji, S. (2011). *Cesiribacter andamanensis* gen. nov., sp. nov., isolated from a soil sample of a mud volcano. *Int J Syst Evol Microbiol* **61**, 1521–1527.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.2. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Xie, C. H. & Yokota, A. (2003). Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.
- Yoon, J., Adachi, K., Park, S., Kasai, H. & Yokota, A. (2011). *Aureibacter tunicatorum* gen. nov., sp. nov., a marine bacterium isolated from a coral reef sea squirt, and description of *Flammeovirgaceae* fam. nov. *Int J Syst Evol Microbiol* **61**, 2342–2347.