International Journal of Systematic and Evolutionary Microbiology (2013), **63**, 2463–2471 **DOI** 10.1099/ijs.0.047134-0

Description of *Tersicoccus phoenicis* gen. nov., sp. nov. isolated from spacecraft assembly clean room environments

Parag Vaishampayan,[1] Christine Moissl-Eichinger,[2] Rüdiger Pukall,[3] Peter Schumann,[3] Cathrin Spröer,[3] Angela Augustus,[4] Anne Hayden Roberts,[4] Greg Namba,[4] Jessica Cisneros,[4] Tina Salmassi[4] and Kasthuri Venkateswaran[1]

Correspondence
Parag Vaishampayan
vaishamp@jpl.nasa.gov

[1]Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA

[2]Institute for Microbiology and Archaea Center, University of Regensburg, Universitaetsstr. 31, 93053 Regensburg, Germany

[3]Leibniz-Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7B, D-38124 Braunschweig, Germany

[4]Department of Biological Sciences, California State University Los Angeles, Los Angeles, CA 90032, USA

Two strains of aerobic, non-motile, Gram-reaction-positive cocci were independently isolated from geographically distinct spacecraft assembly clean room facilities (Kennedy Space Center, Florida, USA and Centre Spatial Guyanais, Kourou, French Guiana). A polyphasic study was carried out to delineate the taxonomic identity of these two isolates (1P05MA^T and KO_PS43). The 16S rRNA gene sequences exhibited a high similarity when compared to each other (100 %) and lower than 96.7 % relatedness with *Arthrobacter crystallopoietes* ATCC 15481^T, *Arthrobacter luteolus* ATCC BAA-272^T, *Arthrobacter tumbae* DSM 16406^T and *Arthrobacter subterraneus* DSM 17585^T. In contrast with previously described *Arthrobacter* species, the novel isolates maintained their coccidal morphology throughout their growth and did not exhibit the rod–coccus life cycle typically observed in nearly all *Arthrobacter* species, except *A. agilis*. The distinct taxonomic identity of the novel isolates was confirmed based on their unique cell-wall peptidoglycan type (A.11.20; Lys-Ser-Ala_2) and polar lipid profile (presence of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, an unknown phospholipid and two unknown glycolipids). The G+C content of the genomic DNA was 70.6 mol%. The novel strains revealed MK-9(H_2) and MK-8(H_2) as dominant menaquinones and exhibited fatty acid profiles consisting of major amounts of anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ and moderate amounts of iso-$C_{15:0}$ discriminating them again from closely related *Arthrobacter* species. Based on these observations, the authors propose that strains 1P05MA^T and KO_PS43 be assigned into a separate genus *Tersicoccus* gen. nov. For this new taxon, comprising strains 1P05MA^T and KO_PS43, we propose the name *Tersicoccus phoenicis* gen. nov., sp. nov. (the type species of *Tersicoccus*), represented by the type strain *Tersicoccus phoenicis* 1P05MA^T (=NRRL B-59547^T=DSM 30849^T).

The inadvertent introduction of terrestrial micro-organisms to extraterrestrial environments could seriously jeopardize the scientific integrity of the life detection missions, and hence is a serious concern for NASA's planetary protection efforts (Rummel, 2001). Consequently, it is important to assemble spacecraft hardware in certified clean room facilities and monitor the biological burden on the surfaces of spacecraft and associated clean room surfaces. Though the extreme conditions of spacecraft assembly clean rooms are effective in reducing the overall microbial load, certain resistant micro-organisms persist, namely those capable of tolerating desiccation, wide ranges of pH, temperature and salt-concentration and exposure to UV light or hydrogen

Abbreviations: DPG, diphosphatidylglycerol; FAME, fatty acid methyl ester; PI, phosphatidylinositol; PG, phosphatidylglycerol.

The GenBank accession numbers for the 16S rRNA gene sequences of *Tersicoccus phoenicis* 1P05MA^T and KO_PS43 are EU977596 and GQ497940, respectively.

Supplementary files are available with the online version of this paper.

peroxide (Ghosh *et al.*, 2010; Kempf *et al.*, 2005; Newcombe *et al.*, 2005; Puleo *et al.*, 1978). The recurrent isolation of extremotolerant bacteria emphasizes the possibility of these micro-organisms gaining access to spacecraft components and ultimately being transferred to extraterrestrial environments, which are potential targets of (future) life-detection missions.

Two strains of a novel non-spore-forming bacterial species were independently isolated from the floor of an ISO 8 (3 520 000 particles >0.5 μm m^{-3}; www.ansi.org) spacecraft assembly clean room at the Kennedy Space Center (KSC), Florida, USA (strain 1P05MA^T) (Ghosh *et al.*, 2010), and at the Centre Spatial Guyanais in the Final Assembly Building, Kourou, French Guiana (strain KO_PS43). More detailed methodology about sample collection and analyses of clean room floor samples are given elsewhere (Ghosh *et al.*, 2010).

Strain 1P05MA^T was obtained on R2A medium (BD), whereas strain KO_PS43 was enriched in liquid anaerobic medium while isolating nitrogen-fixing micro-organisms (Stieglmeier *et al.*, 2009). Both strains were able to grow aerobically on tryptic soy agar (TSA; BD) and subsequent growth characteristics were determined using TSA as growth medium. After 48 h incubation on TSA at 30 °C, colonies were regular edged, yellow in colour, smooth, circular in shape and had a mean diameter of about 1 mm. Using phase-contrast microscopy (Olympus microscope BX-90), cell morphology was monitored; following various incubation time intervals (at 30 °C), in tryptic soy broth (TSB, BD). Both strains yielded positive Gram reactions, as per established procedures (Smibert & Krieg, 1994). The novel isolates exhibited exclusively a coccoid morphology throughout their growth (Fig. S1, available in IJSEM Online). This is a notable difference and can be used as a discriminatory phenotypic trait when distinguishing these novel isolates from previously described *Arthrobacter* species, which reveal a rod and coccus morphology (with the exception of *Arthrobacter agilis*), depending on their physiological status (Chang *et al.*, 2007; Funke *et al.*, 1996; Wauters *et al.*, 2000). The API CORYNE system (bioMérieux) was used for routine biochemical characteristics and API ZYM (bioMérieux) was chosen for additional enzymic characterization (Table 1). All strains tested during this study were inoculated and the data recorded after 1–5 days of incubation were interpreted according to the manufacturer's instructions. Utilization of a broad range of carbon compounds was tested using GP2 MicroPlates (Biolog) for strains KO_PS43 and 1P05MA^T and related type strains of *Arthrobacter* species, according to the manufacturer's instructions. Data for utilization of these carbon sources are indicated in the species description. The ability to grow in varying concentrations of NaCl (0–20 %, w/v) was determined in 1 % Bacto Tryptone supplemented with different amounts of NaCl (Satomi *et al.*, 2006). Growth at varying pH levels was determined as described previously (La Duc *et al.*, 2007). Growth at different temperatures was examined in TSA medium and inoculated cultures were incubated at 4, 10, 20, 30, 37, 40, 45 and 50 °C.

Cellular fatty acid methyl esters (FAME) of strains 1P05MA^T, KO_PS43 and related species of the genus *Arthrobacter* were analysed using the Sherlock MIS (MIDI) system. All the strains were cultivated using TSB (BD) supplemented with Bacto Agar 12.0 g l^{-1} (Difco); incubated for 48 h at 28 °C. The seven strains included in the fatty acid analyses agreed in their growing behaviour and sufficient cells of comparable physiological age could be harvested from the third quadrant of the TSA plates after cultivation under the given conditions. Respiratory quinones were extracted and purified according to established protocol (Collins, 1985) and were analysed by HPLC (Wu *et al.*, 1989).

For analysis of the peptidoglycan structure, cells of strains KO_PS43 and 1P05MA^T were harvested from TSB cultures after 24 h of incubation. Purified cell walls were isolated after disruption of the cells, by shaking with glass beads and subsequent trypsin digestion. The peptidoglycan structure was analysed by using hydrolysates (4 M HCl, 100 °C, 16 h or 0.75 h) of purified cell walls according to published protocols (Schumann, 2011). The amino acids and peptides were separated by two-dimensional ascending thin-layer chromatography on cellulose plates with established solvent systems (Schleifer & Kandler, 1972). The molar ratios of the amino acids in the total hydrolysate (16 h) were determined by GC-MS (320-MS Quadrupole GC/MS, Varian) of *N*-heptafluorobutyryl amino acid isobutyl esters.

The polar lipids of strains 1P05MA^T and KO_PS43 as well as of *Arthrobacter globiformis* DSM 20124^T and *Arthrobacter crystallopoietes* DSM 20117^T were extracted and separated by 2-dimensional TLC as described earlier (Minnikin *et al.*, 1979). To identify spots, ninhydrin reagent, α-naphthol reagent, Zinzadze reagent and molybdophosphoric acid were used (Embley & Wait, 1994). The plates were additionally sprayed with anisaldehyde-sufuric acid (Tindall *et al.*, 2007) for analysis of glycolipids.

Bacterial 16S rRNA genes were amplified using the 27f and 1492r primer set via PCR, as previously described (Satomi *et al.*, 2006). The phylogenetic relationships of the two novel isolates were determined by comparison of individual 16S rRNA gene sequences to existing sequences in public databases (www.ncbi.nlm.nih.gov). For phylogenetic purposes, analyses were performed using the ARB software package (Ludwig *et al.*, 2004). For tree calculations, the 16S rRNA gene sequences of the two isolates were loaded into 'the all-species living tree' project (LTP) tree 104 (Munoz *et al.*, 2011) and compared with all closely related type strains currently available in public databases. The DNA G+C base content was determined by a HPLC method as described earlier (Tóth *et al.*, 2012). DNA–DNA hybridization (DDH) was carried out as described previously (De Ley & Tijtgat, 1970), with some modifications (Huss, *et al.*, 1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a

Table 1. Differential morphological, physiological and biochemical characteristics of *Tersicoccus phoenicis* and related members of the genus *Arthrobacter*

Strain: 1, *T. phoenicis* 1P05MA^T; 2, *A. globiformis* DSM 20124^T; 3, *A. luteolus* ATCC BAA-272^T; 4, *A. crystallopoietes* ATCC 15481^T; 5, *A. subterraneus* DSM 17585^T; 6, *A. tumbae* DSM 16406^T. Morphological, physiological and biochemical data are from this study. Chemotaxonomic data of reference organisms are from earlier reports (Chang *et al.*, 2007; Collins & Jones, 1981; Crombach *et al.*, 1974; De Smedt & De Ley, 1977; Heyrman *et al.*, 2005; Lee *et al.*, 2003; Schleifer & Kandler, 1972; Wauters *et al.*, 2000). +, Positive; −, negative; v, variable. All strains showed positive result for leucine arylamidase, and negative for lipase (C14), valine arylamidase, α-chymotrypsin, trypsin, cystine arylamidase, *N*-acetyl-β-glucosaminidase and α-fucosidase.

Characteristics	1	2	3	4	5	6
Cell morphology	Coccoid	Rod–coccus cycle	Rod–coccus cycle	Rod–coccus cycle	Rod–coccus cycle	Rod–coccus cycle
Motility	−	−	+	−	−	−
Oxidase	+	−	−	−	−	−
Optimum temperature (°C)	30	25	37	20–30	20–30	22–30
Optimum pH	6.0–7.5	8–9	8–9	8–9	5.3–10.5	8–9
Growth at 10 % NaCl	−	+	+	+	+	+
API ZYM						
Alkaline phosphatase	−	−	+	−	−	+
Esterase (C4)	+	+	+	−	v	+
Esterase lipase (C8)	+	−	+	−	−	v
Acid phosphatase	−	−	+	−	−	v
Naphthol-AS-BI-phosphohydrolase	+	−	+	+	+	+
α-Galactosidase	−	+	−	−	−	+
β-Galactosidase	−	−	−	−	v	+
α-Glucosidase	+	+	−	−	+	+
API CORYNE						
Reduction of nitrates	+	−	+	+	+	-
Alkaline phosphatase	−	v	+	v	−	+
β-Glucuronidase	+	−	−	−	−	−
β-Galactosidase	−	+	−	−	+	v
β-Glucosidase (Aesculin)	−	+	−	−	−	−
Gelatin hydrolysis	−	−	−	−	−	+

Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian).

Automated ribotyping was performed for the isolates 1P05MA^T and KO_PS43 by using the DuPont Qualicon RiboPrinter System (Bruce, 1996) with *Pst*I and *Pvu*II as restriction enzymes.

The novel strains described herein were less tolerant to NaCl (up to 2 %, w/v) and were able to grow in the absence of NaCl, while all other closely related species of *Arthrobacter* exhibited growth at high NaCl (6–10 %, w/v) concentrations (Chang *et al.*, 2007; Heyrman *et al.*, 2005). Both the novel strains showed growth at pH 6 and 7.5 but not at pH 3 and 9. The novel strain showed optimum growth at 30 °C after incubation for 48 h. No growth was observed at temperatures below 10 °C or above 40 °C. Table 1 summarizes the physiological and biochemical traits of all the strains examined in this study. Prominent discriminative features not shared between the novel species and related *Arthrobacter* species are: a) β-glucuronidase, b) inability to grow at 10 % (w/v) NaCl, c) absence of rod–coccus life cycle and d) presence of oxidase.

Strain 1P05MA^T exhibited a FAME profile consisting of major amounts of anteiso-$C_{15:0}$ (~43 %) and anteiso-$C_{17:0}$ (~34 %) and moderate amounts of iso-$C_{15:0}$ (~10 %). No significant differences were observed when the cellular fatty acid profile was compared to this of strain KO_PS43, supporting their affiliation to a single species. When compared to the FAME profiles of members of the genus *Arthrobacter*, the novel strains produced significantly higher amounts of anteiso-$C_{17:0}$ and less iso-$C_{15:0}$ (Table 2). The novel strains described here have MK-9(H_2) and MK-8(H_2) as the major menaquinones (average 53 and 37.5 %, respectively) along with MK-10(H_2), MK-9 and MK-8 (average 3.5, 3.0 and 2.2 %, respectively). However, MK-9(H_2) or MK-8, MK-9 was abundant in *Arthrobacter* species, MK-9(H_2), MK-7(H_2), MK-8(H_2) in *Citricoccus* species, and MK-8 and MK-8(H_2) or MK-8(H_2) was reported in *Micrococcus* species (Tables 3 and 4). The amino acids lysine, alanine, serine and glutamic acid were detected in the total peptidoglycan hydrolysate (16 h) in a

Table 2. Fatty acid analysis of *Tersicoccus phoenicis* sp. nov. and related species of the genus *Arthrobacter*

The numbers in the table represent percentage (%) of the fatty acid composition. 1, *T. phoenicis* 1P05MA^T; 2, *T. phoenicis* KO_PS4; 3, *A. globiformis* DSM 20124^T; 4, *A. luteolus* ATCC BAA-272^T; 5, *A. crystallopoietes* ATCC 15481^T; 6, *A. subterraneus* DSM 17585^T; 7, *A. tumbae* DSM 16406^T (all data are from this study).

Fatty acid	1	2	3	4	5	6	7
iso-$C_{14:0}$	0.23	0.26	3.97	2.11	0.6	0.69	2.81
$C_{14:0}$	0.12	0.12	1.85	0.99	0.46	0.72	0.35
iso-$C_{15:0}$	9.94	7.46	20.98	23.26	1.66	32.57	22.97
anteiso-$C_{15:0}$	43.01	50.38	47.97	65.19	83.89	26.59	50.51
iso-$C_{16:1}$	–	–	1.26	–	0.57	1.79	1.27
iso-$C_{16:0}$	6.07	5.91	10.04	3.77	3.24	4.11	8.54
$C_{16:0}$	0.99	0.96	2.45	0.81	1.03	3.06	0.62
anteiso-$C_{17:1\omega9c}$	–	–	1.16	–	1.36	5.58	2.05
iso-$C_{17:0}$	5.17	2.46	1.76	0.4	–	5.38	1.37
anteiso-$C_{17:0}$	34.48	32.46	5.75	2.93	6.13	9.53	7.57

molar ratio of approximately 1.2 : 4.1 : 0.7 : 1.0, respectively. Dinitrophenylation revealed that alanine represented the *N*-terminus of the interpeptide bridge. From these data and from the occurrence of the peptides L-Ala-D-Glu, L-Lys-L-Ser, D-Ala- L-Lys-L-Ser and L-Lys-D-Ala in the partial hydrolysate (0.75 h) it was concluded that both strains KO_PS43 and 1P05MA^T show the peptidoglycan type A3α L-Lys-L-Ser-L-Ala_2 (A11.20; http://www.peptidoglycan-types.info; (Schleifer & Kandler, 1972; Schumann, 2011). The closest relatives of these novel isolates from the genus *Arthrobacter* have completely different peptidoglycan types. Within the suborder *Micrococcineae*, a peptidoglycan with the same interpeptide bridge was found only in *Sinomonas atrocyanea* ATCC 13752^T (Zhou *et al.*, 2009; Schleifer & Kandler, 1972).

Strains 1P05MA^T and KO_PS43 displayed identical polar lipid patterns (not shown) containing phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), two unknown glycolipids, one unknown phospholipid and one unknown lipid. The glycolipids produced red-purple spots with α-naphthol reagent and gave rise to green spots with anisaldehyde-sulfuric acid reagent indicating the possible presence of mannose and/or galactose in these lipids. When compared to the polar lipid patterns of *Arthrobacter globiformis* DSM 20124^T and *Arthrobacter crystallopoietes* DSM 20117^T, the less polar glycolipid (GL2) of strain 1P05MA^T agreed in its staining behaviour and chromatographic mobility to either digalactosyl diacylglycerol or dimannosyl diacylglycerol reported for these *Arthrobacter* species (Collins *et al.*, 1982; Shaw & Stead, 1971) and the more polar glycolipid (GL3) to a triglycosyl diacylglycerol. However, strain 1P05MA^T did not contain a glycolipid with the chromatographic features of a monogalactosyl diacylglycerol (GL1), which was found in *Arthrobacter globiformis* DSM 20124^T, and *Arthrobacter crystallopoietes* DSM 20117^T (Fig. S2; Shaw & Stead, 1971).

Strain 1P05MA^T and KO_PS43 exhibited high 16S rRNA gene sequence similarity (100 %) between each other and are most closely associated to *Arthrobacter crystallopoietes* ATCC 15481^T, *Arthrobacter luteolus* ATCC BAA-272^T, *Arthrobacter tumbae* DSM 16406^T and *Arthrobacter subterraneus* DSM 17585^T (~96 %). All other *Arthrobacter* species revealed a lower sequence similarity including the

Table 3. Differential characteristics of *Tersicoccus* gen. nov. and related genera (except *Arthrobacter*)

Data for reference taxa were taken from Stackebrandt *et al.* (1995), Wieser *et al.* (2002), Altenburger *et al.* (2002) and Zhou *et al.* (2009, 2012). PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; GL, unknown glycolipid(s); PL, unknown phospolipid(s).

Characteristic	*Tersicoccus*	*Kocuria*	*Micrococcus*	*Sinomonas*	*Citricoccus*
Cell morphology	Coccoid	Coccoid	Coccoid	Rod–coccus cycle	Coccoid
Peptidoglycan type	A3α	A3α	A2/A4α	A3α	A4α
DNA G+C content (mol%)	70.6	66-75	66-76	66.9-71.8	64-68
Predominant menaquinone(s)	MK-9(H_2) and MK-8(H_2)	MK-7(H_2), MK-8(H_2)	MK-8 and MK-8(H_2) or MK-8(H_2) only	MK-9(H_2), MK-8(H_2), MK-10(H_2)	MK-9(H_2), MK-7(H_2), MK-8(H_2)
Polar lipids	DPG, PG, PI, PL, 2GL	DPG, PG, PI, PL, GL	DPG, PG, PI, PL, GL	DPG, PG, PI, 2GL	DPG, PG, PI, PL, GL
Predominant fatty acids	ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	ai-$C_{15:0}$, i-$C_{15:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$, i-$C_{15:0}$

Table 4. Combinations of peptidoglycan structures and predominant menaquinones in the genus *Tersicoccus* gen. nov. and in rRNA clusters, subclades and groups of the genus *Arthrobacter* as defined by Busse *et al.* (2012)

	Peptidoglycan type*	Peptidoglycan structure	Predominant menaquinones
Tersicoccus gen. nov.	A11.20	L-Lys-L-Ser-L-Ala_2	MK-9(H_2), MK-8(H_2)
Arthrobacter sensu stricto (rRNA cluster 1)†	A11.4 - A11.7	L-Lys-L-Ala_{1-4}	MK-9(H_2)
Arthrobacter rRNA cluster 2	A11.17	L-Lys-L-Ala-L-Thr-L-Ala	MK-9(H_2)
Arthrobacter rRNA cluster 3	A11.23	L-Lys-L-Ser-L-Thr-L-Ala	MK-9(H_2) in some species additionally MK-9, and/or MK-8
Arthrobacter rRNA cluster 4	A11.35	L-Lys-L-Ala–Glu	MK-8, MK-9
Arthrobacter subclade I	A11.54	L-Lys-L-Glu	MK-9, MK-8, MK-10
Arthrobacter sublade II	A11.27 - A11.28	L-Lys-L-Thr-L-Ala_{2-3}	MK-9(H_2), MK-10(H_2)
Arthrobacter subclade III	A11.27	L-Thr-L-Ala_2	MK-9(H_2), MK-8(H_2)
Arthrobacter subclade IV	A11.4 – A11.7	L-Lys-L-Ala_{1-4}	MK-9(H_2)
	A11.25	L-Lys-L-Thr-L-Ala	
	A11.28	L-Lys-L-Thr-L-Ala_3	
Arthrobacter pigmenti/ castelli group	A11.7		MK-9(H_2)
	-	L-Lys-L-Ala_4 Lys-Ala-Ser-Ala_3	
Arthrobacter albus/ cumminsii group			MK-8(H_2)
	A11.35	L-Lys-L-Ala-L-Glu	
	A11.58	L-Lys-L-Ser-L-Glu	
Arthrobacter species actually not assigned to any group	A11.35	L-Lys-L-Ala-L-Glu	MK-9(H_2)
	A11.41	L-Lys-Gly-L-Ala_3	MK-9(H_2)
	A11.60	L-Lys-D-Asp	-
	-	L-Lys-L-Ala2-L-Glu_{2-3}-L-Ala-(Gly)	MK-9(H_2), MK-8(H_2)

*Nomenclature according to Schumann (2011).

†The assignment of the various *Arthrobacter* species to each rRNA cluster, subclade or group is listed in Table S1.

type species *Arthrobacter globiformis* (95.8 %). Similarly, the type species of the genera *Micrococcus* (94.3 %), *Citricoccus*, *Kocuria* and *Sinomonas* (<95 %) exhibited very low similarity with their 16S rRNA gene sequences. As shown in Fig. 1, the strains 1P05MA^T and KO_PS43 formed a distinct cluster within the *Arthrobacter* species. Tree calculations based on maximum-parsimony (Fig. 1) and neighbour joining (Fig. S3) confirmed the isolated position of the two strains within the genus *Arthrobacter*. All calculations were applied with termini-filter and with or without bacteria-filter. The heterogeneity of the genus *Arthrobacter* and the separate position of *Tersicoccus* are also illustrated in the unrooted phylogenetic network tree based on 16S rRNA gene sequence analysis, but indicating the peptidoglycan types of the various groups related to the genus *Arthrobacter* in addition (Fig. S4).

The DNA G+C content of strain 1P05MA^T was 70.6 mol%. The DDH results showed that strains 1P05MA^T and KO_PS43 were closely related to each other (81 % DNA relatedness), indicating that these novel strains were indeed members of the same species. However, the RiboPrinting patterns of both strains are different and indicate that these strains are not members of a clone (Fig. S5).

Phylogenetic analyses based on the 16S rRNA gene sequences of strains 1P05MA^T and KO_PS43 portray this assemblage as a distinct sublineage, having sequence divergence of >4 % from the closely related *Arthrobacter* species and other related genera. Due to the high divergences in the 16S rRNA gene sequence, DDH was not performed for the novel strains and related *Arthrobacter* species, since such low similarity values generally correlate with low DNA–DNA hybridization values (Stackebrandt & Goebel, 1994). Strains 1P05MA^T and KO_PS43 represent a distinct clade separated from all clusters of *Arthrobacter* species (Fig. 1). Recently Busse *et al.* (2012) have divided the genus *Arthrobacter* into different groups based on 16S rRNA gene sequence similarity and chemotaxonomic features. The tree provided in Fig. S4 clearly demonstrates the complex intrageneric structure of *Arthrobacter* and the heterogeneity in the peptidoglycan types, supporting the views that *Arthrobacter* needs to be dissected into different genera and that the strains 1P05MA^T and KO_PS43 should be affiliated to a separate genus. The peptidoglycan type and menaquinones of strain 1P05MA^T are compared with those of the *Arthrobacter* groups of Busse *et al.* (2012) in Table 4. The peptidoglycan

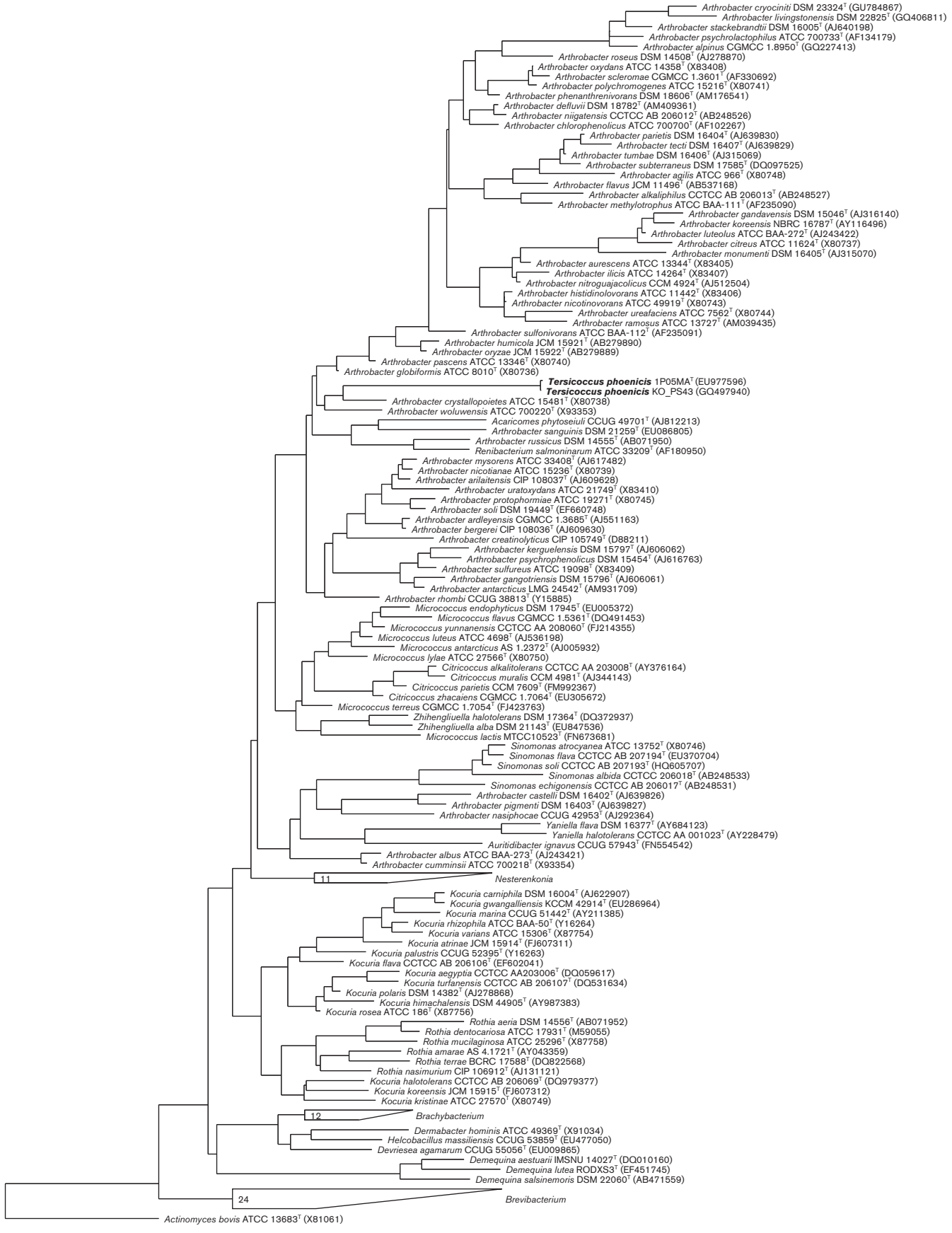

Fig. 1. Phylogenetic analysis based on a Maximum-likelihood algorithm showing position of strains of *Tersicoccus phoenicis* with their closest relatives. Bar, 10 % nucleotide substitutions.

type A11.20 L-Lys-L-Ser-L-Ala_2 in combination with the menaquinones MK-9(H_2) and MK-8(H_2) are unique characteristics differentiating strain 1P05MA^T from all members of the genus *Arthrobacter*. The invariably coccoid morphology and the positive oxidase reaction belong to the numerous morphological, physiological and biochemical features which differentiate strain 1P05MA^T from the five most related type strains of the genus *Arthrobacter* (Table 1). The two n isolates show similar fatty acid profiles to one another but differ in lower amounts of iso-$C_{15:0}$ from the type strains of *A. globiformis* and *A. luteolus* and remarkably higher amounts of anteiso-$C_{17:0}$ from all most related *Arthrobacter* type strains (Table 2).

When compared to the distantly related genera *Kocuria*, *Micrococcus*, *Citricoccus* and *Sinomonas* strain 1P05MA^T can be easily distinguished from the first three genera by peptidoglycan type and predominating menaquinones in their combination (Table 3). The highest agreement in chemotaxonomic characteristics was found with the genus *Sinomonas*. Though peptidoglycan structure and predominating menaquinone were the same, the additional occurrence of an unkown phospholipid and lack of MK-10(H_2) and i-$C_{15:0}$ among the predominating menaquinones and fatty acids may serve in combination with the coccoid morphology for differentiation of strain 1P05MA^T from the genus *Sinomonas*.

The discriminatory phylogenetic inference was supported by phenotypic differences of the new isolates (Tables 1–4) that segregate strains 1P05MA^T and KO_PS43 from related *Arthrobacter* species and other related taxa. Coupled with their deep-branching molecular phylogeny, the differences in phenotypic characteristics advocate a novel genus status for these strains. On the basis of distinguishing biochemical, chemotaxonomic and genotypic attributes, we propose to allocate strains 1P05MA^T and KO_PS43 into a novel genus designated *Tersicoccus* gen. nov. We propose the name *Tersicoccus phoenicis* gen. nov., sp. nov. for the novel genus and species combination.

Description of *Tersicoccus* gen. nov.

Tersicoccus [Ter.si.coc'cus. L. part. adj. *tersus* (from L. v. *tergeo*) clean; N.L. masc. n. *coccus* (from Gr. n. *kokkos* a grain or berry) *coccus*; N.L. masc. n. *Tersicoccus* clean coccus, intended to mean isolated from clean rooms].

Cells are Gram-reaction-positive, non-spore-forming, non-motile cocci. Growth occurs between 30–55 °C and in the presence of up to 2 % NaCl (w/v). The quinone system consists of MK-8(H_2) and MK-9(H_2) as the most prevalent compounds. The polar lipid profile consists of the major compounds DPG and PG, moderate amounts of PI and two unknown glycolipids as well as an unknown phospholipid in trace amounts. Fatty acid profiles consist largely of anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ acids along with moderate amounts of iso-$C_{15:0}$. Cell wall peptidoglycan type is Lys-Ser-Ala_2. The DNA G+C content is approximately 71 mol%. The type species is *Tersicoccus phoenicis*.

Description of *Tersicoccus phoenicis* sp. nov.

Tersicoccus phoenicis (phoe′ni.cis. L. gen. n. *phoenicis* of phoenix, isolated from the surface of the Mars Phoenix spacecraft assembly facility).

These novel strains exhibit smooth, opaque, yellow, round colonies with entire margins and bear a mean diameter of about 1 mm after 48 h of incubation at 30 °C on TSA agar. They do not exhibit rod–coccus life cycle. Cells stain Gram-reaction-positive, and are non-motile cocci. The novel strains grew optimally at 30 °C (no growth below 10 °C or higher than 65 °C), in the presence of 0–2 % (w/v) NaCl, and in a pH range of 6.0–7.5.

Cells are strictly aerobic and oxidase-positive. Cells exhibit positive reactions for nitrate reduction, β-glucuronidase, α-glucosidase, pyrazinamidase and negative reactions are obtained for urease, gelatinase, pyrrolidonyl pyrazinamidase, parylamidase, phosphatase, β-galactosidase, *N*-acetyl-β-glucosaminidase, β-glucosidase (aesculin) and fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, and glycogen. Enzyme activity is detected for esterase (C4), esterase lipase (C8), α-chymotrypsin (weak), naphthol-AS-BI-phosphohydrolase (weak), α-glucosidase (weak). No activity is detected for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, α-galactosidase, β-Glucosidase, α-mannosidase, α-fucosidase, phosphoamidase, and phosphoamidase. Dextrin, Tween 40, Tween 80, D-fructose, D-gluconic acid, α-D-glucose, maltose, maltotriose, D-mannitol, D-mannose, melezitose, palatinose, raffinose (weak), D-ribose, D-sorbitol (weak), trehalose, D-xylose, β-hydroxybutyric acid, L-lactic acid, pyruvic acid methyl ester, pyruvic acid, glycerol and uridine are utilized in the Biolog GP2 test plate. Major whole-cell fatty acids include anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$, while cell wall peptidoglycan is type Lys-Ser-Ala_2.

The type strain, strain 1P05MA^T (=NRRL B-59547^T=DSM 30849^T), was isolated from the Payload Hazardous Servicing Facility (PHSF) at Kennedy Space Center, FL, USA. The DNA G+C content of the type strain is 70.6 mol%

Acknowledgements

The research described in this publication was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. Part of this research was funded by a 2007 NRA ROSES grant to K. V.,

at California State University Los Angeles funded by NAI-MIRS grant to T. S. and at the Regensburg University (CME) under a contract with the European Space Agency (no. 20508/07/NL/EK). The authors extend thanks to Shariff Osman (JPL) and Petra Schwendner (DLR) for sampling and cultivation as well as to Anika Wasner, Melanie Duckstein, Bettina Sträubler (all DSMZ) for excellent technical assistance.

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