Rothia aerea sp. nov., *Rhodococcus baikonurensis* sp. nov. and *Arthrobacter russicus* sp. nov., isolated from air in the Russian space laboratory Mir

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Four Gram-positive bacteria, strains A1-17B^T, A1-22^T, A1-3^T and A1-8, isolated from the air in the Russian space laboratory Mir, were subjected to a polyphasic taxonomic study. Phylogenetic analysis of the bacteria based on their 16S rDNA sequence showed that they belong to the genera *Rothia* (A1-17B^T), *Rhodococcus* (A1-22^T) and *Arthrobacter* (A1-3^T and A1-8). Morphological, physiological, chemotaxonomic and genomic characteristics supported the assignments of these strains to these genera, but they could not be classified as any existing species within each respective genus. 16S rDNA similarity values between strain A1-17B^T and its neighbours, *Rothia dentocariosa* genomovar II, *Rothia dentocariosa*, *Rothia mucilaginosa* and *Rothia nasimurium*, were respectively 99.8, 98.0, 96.4 and 95.4 %. Polyphasic taxonomic evidence indicated that strain A1-17B^T should be categorized together with the unofficially named *Rothia dentocariosa* genomovar II, but clearly differentiated them from the established species of the genus *Rothia*. Strain A1-22^T formed a coherent cluster with *Rhodococcus erythropolis*, *Rhodococcus globerulus*, *Rhodococcus marinonascens* and *Rhodococcus percolatus* in 16S rDNA sequence analysis, but DNA–DNA relatedness values were only 45.5, 35.3, 18.9 and 21.9 %. Strains A1-3^T and A1-8 shared 99.9 % 16S rDNA sequence similarity, and strain A1-3^T showed the highest level of 16S rDNA similarity, 96.6 %, to *Arthrobacter polychromogenes*. Contrasting biochemical characteristics were also identified. Finally, as a result of the polyphasic taxonomic study, three of the strains are proposed as type strains of novel species: *Rothia aerea* sp. nov. (A1-17B^T=GTC 867^T=JCM 11412^T=DSM 14556^T), *Rhodococcus baikonurensis* sp. nov. (A1-22^T=GTC 1041^T=JCM 11411^T=DSM 44587^T) and *Arthrobacter russicus* sp. nov. (A1-3^T=GTC 863^T=JCM 11414^T=DSM 14555^T).

INTRODUCTION

Decelle & Taylor (1976) reported that in-flight cross-contamination and build-up of pathogens were noted in the Apollo space station. The space station is a special environment with reduced gravity, and crews must live in a completely closed environment for extended periods of time. Because of the many unfavourable effects of space flight and mental stress, astronauts may suffer weakened

immunocompetence and may even become infected by opportunistic pathogens (Criswell-Hudak, 1991). It is therefore essential to understand and control the microbial population in these environments. Bacteria were isolated from air and condensation water samples from the Russian space station Mir in 1997 and the bacterial population was analysed. Previous investigation had shown that bacteria from air samples were mainly Gram-positive, and most of the strains were opportunistic pathogens (Kawamura *et al.*, 2001). A polyphasic taxonomic study showed that seven strains, belonging to six species, failed to match any established species. Three Gram-negative species of these isolates have been described elsewhere (Li *et al.*, 2003, 2004). The taxonomic status of the four Gram-positive isolates was investigated in detail here.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Arthrobacter russicus* sp. nov. A1-3^T, *Rothia aerea* sp. nov. A1-22^T and *Rhodococcus baikonurensis* sp. nov. A1-17B^T are AB071950–AB071952.

Gram-positive bacteria have stronger resistance to dry conditions than do Gram-negative bacteria because of their firm cell-wall structure (Neidhardt, 1990). All four Gram-positive strains were isolated from air samples. These bacteria, drifting in the air of the space station, may become an infectious source and threaten the health of space-station astronauts, especially when astronauts have weakened immunocompetence. The four Gram-positive isolates were affiliated with the genera *Rothia*, *Rhodococcus* and *Arthrobacter*. As a result of polyphasic taxonomic study, these isolates should now be classified as the novel species *Rothia aeria* sp. nov., *Rhodococcus baikonurensis* sp. nov. and *Arthrobacter russicus* sp. nov.

METHODS

Sample collection, morphology, physiology and DNA extraction.

Air samples from the living environment of the Mir space station were collected and filtered and filters were stored as described by Li *et al.* (2004). After transfer to the laboratory, samples were cultured as described by Li *et al.* (2004) with incubation at 30 °C. Biochemical characterization of the isolates was performed using API CORYNE (API bioMérieux) and Biolog GP2 MicroPlate assay (Biolog). Oxidase activity was determined with oxidase test strips (Eiken Chemical). DNA was extracted as described by Ezaki *et al.* (1994).

Chemotaxonomic characterization. Diamino acids were analysed by TLC as described by Kawamura *et al.* (1995). Other cell-wall amino acid analysis was performed as described by Komagata & Suzuki (1987). Briefly, bacteria were suspended in 10 ml 10% trichloroacetic acid (w/v) and boiled for 30 min. The lysed bacteria were separated by centrifugation and the precipitate was then washed twice with distilled water and concentrated down at 10 000 r.p.m. for 10 min, resuspended in 10 ml PBS (0.145 M sodium chloride, 5 mM monobasic sodium phosphate, 8 mM dibasic sodium phosphate, pH 7.9) containing trypsin (2 mg ml⁻¹) and incubated at 37 °C for 3 h. The precipitate was collected after centrifugation and washed twice with distilled water. It was then freeze-dried. Approximately 1 mg cell wall was hydrolysed for 18 h at 100 °C in 1 ml 6 M HCl. The hydrolysate was dried in a rotary evaporator and dissolved in 100 µl distilled water. Amino acids were mixed with 20 µl phenyl isothiocyanate solution (Wako) (ethanol/distilled water/trimethylamine/phenyl isothiocyanate, 7:1:1:1 by vol.) at room temperature for 20 min and analysed by HPLC (Hitachi UV detector L-7400) equipped with a Wakopak WS-PTC (4 × 200 mm) column.

Cellular fatty-acid compositions were measured using the Sherlock Microbial Identification system (MIDI) and method as described by Kosako *et al.* (2000). Mycolic acids were extracted and analysed as described by Nishiuchi *et al.* (1999). Polar lipids were identified as described by Minnikin *et al.* (1984). Isoprenoid quinone analysis was performed by reverse-phase TLC as described by Collins *et al.* (1977) and Yano *et al.* (1987).

Analysis of 16S rDNA sequence and DNA base composition.

The 16S rRNA gene was amplified with universal primers (forward: 5'-AGAGTTTGATCMTGGCTCAG-3'; reverse: 5'-ACGGGCGGTG-TGTRC-3') and the PCR products for all isolates were sequenced in both directions. The sequences were analysed and trees generated as described by Li *et al.* (2004).

The G + C content was measured by HPLC as described by Ezaki *et al.* (1990). *Escherichia coli* was used as a standard (G + C content 51.19 mol%).

DNA–DNA hybridization. Quantitative microplate DNA–DNA hybridization for selected strains was carried out as described by Ezaki *et al.* (1988, 1989). Hybridization experiments were carried out under optimal and stringent temperatures calculated from melting temperatures (*T_m*) based on the G + C content of each test strain.

RESULTS AND DISCUSSION

Strain A1-17B^T

The genus *Rothia* was proposed by Georg & Brown (1967) with *Rothia dentocariosa* as the type species. The phenotypic heterogeneity within the species has long been known (Leshner *et al.*, 1974; Schofield & Schaal, 1981; Fotos *et al.*, 1984), and the existence of a second genomovar was described by Kronvall *et al.* (1998). However, *Rothia dentocariosa* was recognized as the only species of the genus until Collins *et al.* (2000) described a novel species (*Rothia nasimurium*) and reclassified *Stomatococcus mucilaginosus* as *Rothia mucilaginosa*. Fan *et al.* (2002) proposed a further member, *Rothia amarae*.

Strain A1-17B^T, isolated from air samples in the Russian space station Mir, formed a distinct phylogenetic subline and exhibited a specific association with genus *Rothia* (Fig. 1). 16S rDNA sequence similarity values between A1-17B^T and its neighbours, *Rothia dentocariosa* genomovar II, *Rothia dentocariosa*, *Rothia mucilaginosa*, *Rothia amarae* and *Rothia nasimurium*, were respectively 99.8, 98.0, 96.4,

Fig. 1. Phylogenetic position of four Gram-positive isolates from the Mir space station and selected members of the genera *Arthrobacter*, *Rothia* and *Rhodococcus* based on 16S rDNA sequences. Distances were calculated by the neighbour-joining method. Numbers at branch points are bootstrap values (based on 1000 samplings). GenBank accession numbers are given. Bar, 0.01 estimated substitutions per nucleotide position. *E. coli* was used as an outgroup.

96.0 and 95.4%, as determined by CLUSTAL W analysis. Similarity values to other members of the family *Micrococcaceae* were less than 95.0%. Phylogenetic data showed that strain A1-17B^T is a member of the genus *Rothia*. The G+C content is 57.8 mol%, which is within the range of 47–59 mol% observed in the genus (Bergan & Kocur, 1982; Gerencser & Bowden, 1986). This strain also showed morphological similarity to members of the genus *Rothia* (Georg & Brown, 1967) (see species description below).

TLC analysis indicated that the isolated strains did not contain diaminopimelic acid in the cell wall, which is consistent with *Rothia dentocariosa* (Georg & Brown, 1967). HPLC analysis of the peptidoglycan showed that the amino acids glutamic acid, alanine and lysine occur in a molar ratio of 1:3:1; these results suggest that the interpeptide bridge contains alanine and that the peptidoglycan type is A3 α -type (Schleifer & Kandler, 1972). The

predominant isoprenoid quinone is menaquinone with seven isoprenoid units (MK-7), which is consistent with other related species in the genus *Rothia* (Table 1). Examination of the cellular fatty acids of A1-17B^T revealed that the acids are predominantly of the iso- and anteiso-methyl-branching types. This is also similar to the composition of other species of *Rothia* (Table 1). The predominant polar lipids were phosphatidylglycerol and cardiolipin, with phosphatidylinositol as a minor component.

Strain A1-17B^T and *Rothia dentocariosa* genomovar II (CCUG 25688, CCUG 33543) showed the following common biochemical characteristics with *Rothia dentocariosa* and *Rothia mucilaginosa*: positive for nitrate reduction, pyrazinamidase, alanine-phenylalanine-proline arylamidase, α -glucosidase and β -glucosidase activities, hydrolysis of aesculin and acid production from glucose, maltose and sucrose; negative for β -glucuronidase, β -galactosidase,

Table 1. Differential characteristics of *Rothia aerea* sp. nov. A1-17B^T and closely related species of the genus *Rothia*

Strains: 1, *Rothia aerea* sp. nov. A1-17B^T; 2, *Rothia dentocariosa* genomovar II CCUG 25688; 3, *Rothia dentocariosa* genomovar II CCUG 33543; 4, *Rothia dentocariosa* JCM 3067^T; 5, *Rothia mucilaginosa* CCM 2417^T. Data for cellular fatty acids for reference strains are from Collins *et al.* (2000) and Fan *et al.* (2002). Strain A1-17B^T also contains anteiso-13:0, 14:0 and iso-17:0 (each <1%). *Rothia dentocariosa* also contains 15:0 (2.2%). w, Weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Utilization of:					
3-Methyl glucose	+	w	+	—	+
Methyl α -D-glucoside	+	+	+	—	—
Methyl β -D-glucoside	+	+	+	—	+
D-Psicose	+	+	w	—	—
Salicin	+	+	+	—	+
L-Malic acid	—	—	—	+	—
α -Hydroxybutyric acid	+	w	+	+	—
Pyruvic acid	—	—	—	w	+
Succinic acid	—	—	—	+	—
L-Lactic acid	+	+	+	+	—
Methyl pyruvate	—	—	w	+	+
Monomethyl succinate	—	—	—	w	—
2,3-Butanediol	—	—	w	+	+
Glycerol	+	+	+	+	—
DL- α -Glycerol phosphate	—	—	—	—	+
Catalase	+	+	+	+	—
Alkaline phosphatase	—	—	—	—	+
Major menaquinone	MK-7	ND	ND	MK-7	MK-7
Cellular fatty acid composition (%):					
iso-14:0	2.7	3.5	ND	4.0	10.8
iso-15:0 (13-methyl-14:0)	4.5	4.5	ND	3.3	5.9
anteiso-15:0	52.9	51.6	ND	53.3	45.7
iso-16:0 (14-methyl-15:0)	20.3	17.3	ND	14.6	21.6
16:0	1.9	7.1	ND	6.0	9.6
anteiso-17:0 (14-methyl-18:0)	16.0	16.0	ND	16.9	6.4
Relationship to strain A1-17B ^T :					
16S rDNA sequence similarity (%)	(100)	99.8	ND	98.0	96.4
DNA–DNA relatedness (%)	(100)	100.0	100.0	34.1	21.2

N-acetyl- β -glucosaminidase and urease activities and production of acid from ribose, xylose, mannitol, lactose and glycogen. These similar biochemical profiles support their relationship to these taxa. However, some common characteristics of strain A1-17B^T and *Rothia dentocariosa* genomovar II differentiated them from *Rothia dentocariosa* and *Rothia mucilaginosa* (Table 1). The results of the Biolog GP2 assay for strain A1-17B^T are summarized in Table 2.

DNA–DNA hybridization showed that strain A1-17B^T and *Rothia dentocariosa* genomovar II have 100% DNA relatedness, whereas they showed only 34.1 and 21.2% relatedness with their related neighbours *Rothia mucilaginosa* and *Rothia dentocariosa* (Table 1), confirming that this strain represents a taxonomically independent species.

16S rDNA sequence similarity of 99.8% and DNA–DNA relatedness of 100% between strain A1-17B^T and *Rothia dentocariosa* genomovar II indicated that they belong to the same species. Kronvall *et al.* (1998) suggested the possibility that *Rothia dentocariosa* genomovar II is a novel species of the genus. However, *Rothia dentocariosa* genomovar II has not been formally named as it could not be distinguished from authentic *Rothia dentocariosa* using API systems (Kronvall *et al.*, 1998). In this study, strain A1-17B^T, *Rothia dentocariosa* genomovar II and *Rothia dentocariosa* also showed the same biochemical characteristics using the API Coryne system, but the Biolog GP2 system clearly differentiated them biochemically from *Rothia dentocariosa* (Table 1). DNA hybridization data also supported strain A1-17B^T and *Rothia dentocariosa* genomovar II as belonging to the same species and independent from other species of *Rothia*. Based on polyphasic taxonomic analysis, strain A1-17B^T and *Rothia dentocariosa* genomovar II (strains CCUG 25688 and CCUG 33543) clearly merit classification as a novel species within the genus *Rothia*, *Rothia aerea* sp. nov.

Strain A1-22^T

Sequence analysis by the FASTA search system on the DDBJ website showed that strain A1-22^T was phylogenetically most closely related to the genus *Rhodococcus*. The phylogenetic position of strain A1-22^T as analysed by CLUSTAL W software showed that it is closely related to *Rhodococcus erythropolis*, *Rhodococcus globerulus*, *Rhodococcus marinonascens* and *Rhodococcus percolatus*, with respective 16S rDNA sequence similarities of 99.2, 98.9, 97.6 and 97.0% (Fig. 1). Similarity values to other *Rhodococcus* species and species of other genera were less than 97% (data not shown). Phylogenetic analysis supported the affinity of strain A1-22^T to genus *Rhodococcus*.

Strain A1-22^T also showed similar phenotypic characteristics to *Rhodococcus* species (see species description below). Biochemically, strain A1-22^T showed catalase and urease activities, but not oxidase activity, and was able to hydrolyse aesculin but not arbutin. The results of the Biolog GP2 analysis are shown in Table 2. Although strain A1-22^T

showed similar biochemical properties to its closest relative, *Rhodococcus erythropolis*, hydrolysis of arbutin and utilization of *m*-inositol enable them to be differentiated (Table 3).

TLC analysis of strain A1-22^T indicated that the strain contains *meso*-diaminopimelic acid as the major diamino acid in the cell wall, which is a feature of the genus *Rhodococcus* (Goodfellow, 1986). HPLC analysis of the peptidoglycan showed that glycine, alanine and diaminopimelic acid are present in a 1:2:1 molar ratio. These results suggest that the peptidoglycan is A1 γ -type, which is consistent with *Rhodococcus fascians* (Goodfellow, 1984). The predominant isoprenoid quinone is MK-8(H₂), which is consistent with the closely related species of the genus *Rhodococcus* (Goodfellow, 1986).

The cellular fatty acid composition of strain A1-22^T was: 14:0, 5%; *cis*-16:1, 9%; 16:0, 41%; *cis*-18:1, 18%, 18:0, 4%, tuberculostearic acid (10-methyl-18:0), 22% (only components representing $\geq 1\%$ of the total are reported). This composition is similar to that of some *Rhodococcus* species, but the strain can be differentiated from *Rhodococcus erythropolis* based on the content of 16:0 and 19:0 (22 and 10.9% in *Rhodococcus erythropolis*; Yoon *et al.*, 2000). The number of carbons in mycolic acids varies from 22 to 90, compared with the genera *Corynebacterium* (22–28), *Gordonia* (48–66), *Mycobacterium* (66–90), *Nocardia* (46–60) and *Rhodococcus* (32–66) (Goodfellow, 1986, 1992). The mycolic acid carbon chain of strain A1-22^T determined by GLC/MS was 32–42 atoms in length with three double bonds, consistent with the genus *Rhodococcus*, with zero to four double bonds (Goodfellow, 1986). The cellular phospholipids were phosphatidylethanolamine, cardiolipin, phosphatidylinositol and phosphatidylinositol mannosides, again consistent with the properties of the genus *Rhodococcus* (Goodfellow, 1986).

DNA–DNA relatedness values of strain A1-22^T with its closely related neighbours *Rhodococcus erythropolis*, *Rhodococcus globerulus*, *Rhodococcus marinonascens* and *Rhodococcus percolatus* were only 45.5, 35.3, 18.9 and 21.9%. All hybridization rates were below the suggested threshold value ($\sim 70\%$) (Grimont, 1999; Wayne *et al.*, 1987), confirming that this strain represents a genetically independent species.

Based on polyphasic taxonomic results, strain A1-22^T is affiliated with the genus *Rhodococcus*. However, based on its biochemical properties and chemotaxonomic characteristics, strain A1-22^T is different from any established species of the genus. DNA–DNA hybridization data ($< 70\%$) confirmed that strain A1-22^T taxonomically represents an independent species. Thus, we propose the name *Rhodococcus baikonurensis* sp. nov. for this organism.

Strain A1-3^T

Sequence analysis by the FASTA search system on the DDBJ website showed that strains A1-3^T and A1-8 were phylogenetically most closely related to the genus

Table 2. Results of Biolog GP2 assays

Strains: 1, *Rothia aerea* sp. nov. A1-17B^T; 2, *Rothia dentocariosa* genomovar II CCUG 25688; 3, *Rhodococcus baikonurensis* sp. nov. A1-22^T; 4, *Arthrobacter russicus* sp. nov. A1-3^T; 5, *A. russicus* sp. nov. A1-8. W, Weakly positive. All strains reacted positive for α -D-glucose, D-psicose and glycerol. All strains reacted negative for α -cyclodextrin, β -cyclodextrin, glycogen, inulin, mannan, N-acetyl D-glucosamine, N-acetyl D-mannosamine, amygdalin, L-arabinose, D-arabitol, cellobiose, L-fructose, D-galactose, D-galacturonic acid, gentiobiose, m-inositol, α -D-lactose, lactulose, D-mannitol, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, D-raffinose, L-rhamnose, methyl α -D-mannoside, sedoheptulosan, D-sorbitol, stachyose, D-tagatose, xylitol, p-hydroxyphenyl acetic acid, D-xylose, α -ketoglutaric acid, lactamide, D-malic acid, D-alanine, N-acetyl L-glutamic acid, L-alanine, glycyl L-glutamic acid, L-pyroglutamic acid, L-serine, adenosine, 2'-deoxyadenosine, inosine, thymidine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate and DL- α -glycerol phosphate.

Compound	1	2	3	4	5
Dextrin	+	+	—	—	—
Tween 40	—	—	+	+	+
Tween 80	—	—	+	+	+
Arbutin	+	—	—	—	—
D-Fructose	+	W	+	+	+
D-Gluconic acid	—	—	+	—	—
Maltose	+	+	—	—	—
Maltotriose	+	+	—	—	—
D-Mannose	+	+	W	+	+
D-Melezitose	+	+	—	—	—
3-Methyl glucose	+	W	—	—	—
Methyl α -D-glucoside	+	+	—	—	—
Methyl β -D-glucoside	+	+	—	—	—
Palatinose	+	+	—	—	—
D-Ribose	—	—	+	—	—
Salicin	+	+	—	—	—
Sucrose	+	+	—	—	—
D-Trehalose	+	+	—	—	—
Turanose	+	+	—	—	W
Acetic acid	—	—	+	—	—
α -Hydroxybutyric acid	+	W	+	—	—
β -Hydroxybutyric acid	—	—	+	—	—
γ -Hydroxybutyric acid	—	—	+	—	—
α -Ketovaleric acid	—	—	+	—	—
D-Lactic acid methyl ester	—	—	W	—	—
L-Lactic acid	+	+	+	—	—
L-Malic acid	—	—	+	—	—
Methyl pyruvate	—	—	+	+	+
Monomethyl succinate	—	—	+	W	W
Propionic acid	—	—	W	—	—
Pyruvic acid	—	—	+	—	+
Succinamic acid	—	—	W	—	—
Succinic acid	—	—	W	—	—
Alaninamide	—	—	W	—	—
L-Alanyl glycine	—	—	—	+	+
L-Asparagine	—	—	+	—	—
L-Glutamic acid	—	—	W	W	W
Putrescine	—	—	+	+	+
2,3-Butanediol	—	—	W	—	—
Uridine	—	—	—	+	—
Uridine 5'-monophosphate	—	—	—	W	—

Table 3. Differential characteristics of *Rhodococcus baikonurensis* sp. nov. A1-22^T and closely related *Rhodococcus* species

Strains: 1, *Rhodococcus baikonurensis* A1-22^T; 2, *Rhodococcus erythropolis* JCM 3201^T; 3, *Rhodococcus fascians* DSM 20669^T; 4, *Rhodococcus globerulus* JCM 14531^T; 5, *Rhodococcus opacus* DSM 43205^T; 6, *Rhodococcus percolatus* MBS1^T; 7, *Rhodococcus marinonascens* DSM 43752^T. Data were taken from this study, Yoon *et al.* (2000), Klatte *et al.* (1994) and Briglia *et al.* (1996). w, Weakly positive.

Characteristic	1	2	3	4	5	6	7
Hydrolysis of:							
Arbutin	—	+	—	+	—	—	—
Aesculin	+	+	—	+	—	—	+
Utilization of:							
L-Arabinose	—	—	+	—	—	—	—
D-Xylose	—	—	+	—	w	+	—
D-Galactose	—	—	w	—	+	—	—
Lactose	—	—	—	—	+	+	—
Maltose	—	—	—	—	+	+	—
Melezitose	—	—	—	—	+	+	—
D-Melibiose	—	—	—	—	+	+	—
D-Raffinose	—	—	—	—	+	—	—
D-Turanose	—	—	—	—	+	+	—
Arabitol	—	—	+	+	+	+	—
<i>m</i> -Inositol	—	+	—	—	+	+	+
Xylitol	—	—	+	—	+	—	—
Inulin	—	—	—	—	—	—	+
Production of urease	+	+	+	+	+	+	—
Number of carbon atoms in mycolic acids	32–42	30–44	44–51	30–42	48–54	46–54	34–50

Arthrobacter. A tree constructed using the neighbour-joining method depicting the phylogenetic position of strains A1-3^T and A1-8 (Fig. 1) showed that strains A1-3^T and A1-8 were closely related to *Arthrobacter polychromogenes*, *Arthrobacter oxydans* and *Arthrobacter psychrolactophilus*, with respective 16S sequence similarity of 96.6, 96.5 and 95.5 % (Table 4). 16S rDNA sequence similarity values to members of other genera were less than 95 %. Strains A1-3^T and A1-8 exhibited an extremely high 16S rDNA sequence similarity of 99.9 %. These results clearly demonstrated that strains A1-3^T and A1-8 belong to the genus *Arthrobacter*.

Phenotypic and biochemical characteristics of strains A1-3^T and A1-8 (see species description) were consistent with the description of the genus *Arthrobacter* (Keddie *et al.*, 1986). However, strains A1-3^T and A1-8 can be differentiated biochemically from closely related species of the genus *Arthrobacter* (Table 4). The metabolic properties determined from the Biolog GP2 assay are shown in Table 2.

The cellular fatty acid content of strain A1-3^T was as follows (only values ≥ 1 % reported): iso-15:0 (13-methyl-14:0), 1 %; anteiso-15:0 (12-methyl-14:0), 20 %; iso-16:0 (14-methyl-15:0), 4 %; 16:0, 2 %; iso-17:0 (15-methyl-16:0), 1 %; anteiso-17:0 (14-methyl-16:0), 64 %; anteiso-19:0 (16-methyl-18:0), 2 %; 2-OH 14:0, 1 %; and 2-OH 16:0, 4 %. This composition was consistent with that of members of the genus *Arthrobacter*, with anteiso-15:0 predominating. However, unlike many *Arthrobacter* species, including

A. oxydans, *Arthrobacter agilis* and *Arthrobacter roseus* (Reddy *et al.*, 2002; Kodama *et al.*, 1992), strain A1-3^T contained anteiso-19:0, 2-OH 14:0 and 2-OH 16:0. The predominant polar lipids were cardiolipin and phosphatidylinositol, with phosphatidylglycerol as a minor component. This composition is consistent with that of related species of the genus *Arthrobacter* (Reddy *et al.*, 2002). Strains A1-3^T and A1-8 lack diaminopimelic acid in their cell walls. The amino acids glutamic acid, alanine and lysine were detected in a molar ratio of 1:4:1 in the peptidoglycan. These results suggest that the interpeptide contains alanine and the peptidoglycan is A3 α -type (Kodama *et al.*, 1992; Fiedler *et al.*, 1973), consistent with the peptidoglycan type found in species of the *A. polychromogenes* group (Koch *et al.*, 1995). The predominant isoprenoid quinone was MK-9(H₂).

Strains A1-3^T and A1-8 share DNA–DNA relatedness of 100 %, whereas these two strains showed only 9.8 % DNA–DNA relatedness with the nearest neighbour, *A. polychromogenes*. DNA–DNA hybridization is thought to be the superior method for the determination of relationships between bacteria (Stackebrandt & Goebel, 1994). These results demonstrate that strains A1-3^T and A1-8 belong to the same genetic species, which is independent of the established species of the genus *Arthrobacter*.

Phenotypic and chemotaxonomic characteristics of strains A1-3^T and A1-8 were also consistent with the description of the genus *Arthrobacter* and support the conclusion from

Table 4. Differential characteristics of *Arthrobacter russicus* sp. nov. strains A1-3^T and A1-8 and closely related *Arthrobacter* species

Strains: 1, *A. russicus* strains A1-3^T and A1-8; 2, *A. agilis* CCM 2390^T; 3, *A. polychromogenes* DSM 20136^T; 4, *A. psychrolactophilus* ATCC 700733^T; 5, *A. crystallopoietes* ATCC 15481^T; 6, *A. oxydans* ATCC 14358^T; 7, *A. roseus* DSM 14508^T. Data were taken from this study, Loveland-Curtze *et al.* (1999), Funke *et al.* (1996) and Reddy *et al.* (2002). NA, Data not available; W, weakly positive.

Characteristic	1	2	3	4	5	6	7
Colony colour	Cream/white	Red	Blue/green	Yellow	White	Grey/white	Red
Hydrolysis of:							
Aesculin	—	+	+	NA	—	—	—
Tween 80	+	—	—	NA	NA	NA	NA
Urease activity	—	—	—	—	+	+	—
Nitrate reduction	—	—	+	—	+	+	+
Utilization of:							
Inositol	—	—	—	NA	+	+	—
Sorbitol	—	+	—	+	—	+	+
Lactose	—	—	+	+	—	+	+
Xylitol	—	—	—	NA	—	+	NA
Xylose	—	—	—	+	—	+	+
Glucose	+	+	+	+	—	+	+
Maltose	—	—	—	+	—	—	+
Mannitol	—	+	+	NA	—	—	+
Sucrose	—	W	+	+	—	+	+
Growth at 37 °C	—	—	+	—	NA	+	—
16S rDNA sequence similarity (%) to strain A1-3 ^T	99.9*	95	96.6	96	95.5	96.5	95.3
Peptidoglycan type	Lys-Ala ₂	Lys-Thr-Ala ₃	Lys-Ser-Thr-Ala	Lys-Ala ₃	Lys-Ala	Lys-Ser-Thr-Ala	Lys-Gly-Ala ₃

*Similarity between sequences of strains A1-3^T and A1-8.

the 16S rRNA alignment that these two strains belong to the genus *Arthrobacter*. However, biochemical characteristics and cellular fatty acid composition and DNA hybridization data sharply differentiate them from the closely related species *A. polychromogenes*. Therefore, the name *Arthrobacter russicus* sp. nov. is proposed for this species.

Description of *Rothia aeria* sp. nov.

Rothia aeria (aer'i.a. L. fem. adj. *aeria* of the air, referring to the isolation of the type strain from the air inside the Mir space station).

Grows well under aerobic conditions at 30 °C on BHI agar plates. Gram-positive cells appear coccoid, cocco-bacillary or filamentous. Young colonies are creamy white and smooth. Mature colonies are rough, dry, folded and convex and adhere to the agar medium such that they are not easily scraped off. Biochemical characteristics are summarized in Tables 1 and 2. Cell-wall peptidoglycan is A3 α -type. Predominant isoprenoid quinone is MK-7. Major cellular fatty acids are anteiso-15:0 (12-methyl-14:0), iso-16:0 (14-methyl-15:0) and anteiso-17:0 (14-methyl-18:0). Major polar lipids are phosphatidylglycerol and cardiolipin. The G + C content of the type strain is 57.8 mol%.

The type strain, A1-17B^T (=GTC 867^T=JCM 11412^T=DSM 14556^T), was isolated from an air sample from the Russian space station Mir. This species includes strains formerly categorized as *Rothia dentocariosa* genomovar II.

Description of *Rhodococcus baikonurensis* sp. nov.

Rhodococcus baikonurensis (bai.kon.ur.en'sis. N.L. masc. adj. *baikonurensis* of Baikonur, the town in Kazakhstan where the Mir space station was launched).

Grows well under aerobic conditions at 30 °C on BHI agar plates. A mixture of Gram-positive rods and cocci show elementary branching. Colonies are smooth, opaque and slightly pink. Biochemical characteristics are summarized in Tables 2 and 3. Diamino acid in the peptidoglycan is meso-diaminopimelic acid. Cell-wall peptidoglycan is A1 γ -type. Predominant isoprenoid quinone is MK-8(H₂). Major cellular fatty acids are 16:0, *cis*-18:1 and tuberculostearic acid (10-methyl-18:0). Mycolic acids contain 46–54 carbon atoms. Phospholipid composition is phosphatidylethanolamine, cardiolipin, phosphatidylinositol and phosphatidyl-inositol mannosides. The G + C content of the type strain is 55.5 mol%.

The type strain, A1-22^T (=GTC 1041^T=JCM 11411^T=DSM 44587^T), was isolated from an air sample from the Russian space station Mir.

Description of *Arthrobacter russicus* sp. nov.

Arthrobacter russicus [rus'sic.us. L. masc. adj. *russicus* pertaining to Russia (Russian space station)].

Grows well under aerobic conditions at 30 °C on BHI agar plates, but unable to grow at 37 °C. Gram-positive, non-motile, irregular rods, about 1.3–3.6 µm long and 0.5–0.9 µm wide. Circular, smooth and creamy colonies grow to a diameter of about 1.5 mm after 24 h. Biochemical characteristics are summarized in Tables 2 and 4. Cell-wall peptidoglycan is A3α-type. Predominant isoprenoid quinone is MK-9(H₂). Major cellular fatty acids are anteiso-15:0 (12-methyl-14:0) and anteiso-17:0 (14-methyl-16:0). Predominant polar lipids are cardiolipin and phosphatidylinositol. The G+C content of the type strain is 65.5 mol%.

The type strain, A1-3^T (=GTC 863^T=JCM 11414^T=DSM 14555^T), was isolated from an air sample from the Russian space station Mir.

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