Proposal of *Sinomonas flava* gen. nov., sp. nov., and description of *Sinomonas atrocyanea* comb. nov. to accommodate *Arthrobacter atrocyaneus*

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A novel actinomycete strain, designated CW 108^T, was isolated from a forest soil in Anhui Province, China. The cells were strictly aerobic, non-motile, bent rods. The strain grew optimally at 30–37 °C and pH 6.0–8.0. Chemotaxonomically, the peptidoglycan type was A3 α , the cell-wall sugars contained galactose, mannose and ribose, the polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylmonomethylethanolamine, the major fatty acids were ai-C_{15:0}, i-C_{15:0} and ai-C_{17:0} and the predominant respiratory quinone was MK-9(H₂), with a moderate amount of MK-8(H₂) and a minor amount of MK-6(H₂). The G + C content of the genomic DNA was 71.1 mol%. Phylogenetic analysis showed that strain CW 108^T formed a deeply separated lineage in the family *Micrococcaceae* with *Arthrobacter atrocyaneus* DSM 20127^T. The 16S rRNA gene sequence similarity between strain CW 108^T and *Arthrobacter atrocyaneus* DSM 20127^T was 99.4%; sequence similarities between these strains and representatives of other species in the family *Micrococcaceae* with validly published names were lower than 95.2%. DNA–DNA hybridization showed a level of relatedness of 52.2% between the two neighbours. Based on the results of our polyphasic taxonomic study, strain CW 108^T and *Arthrobacter atrocyaneus* DSM 20127^T should be assigned to two different species of a novel genus within the family *Micrococcaceae*, for which the names *Sinomonas flava* gen. nov., sp. nov. and *Sinomonas atrocyanea* comb. nov. are proposed. The type strain of *Sinomonas flava* is CW 108^T (=LMG 24447^T =KCTC 19388^T =CCTCC AB 207194^T) and the type strain of *Sinomonas atrocyanea* is DSM 20127^T =JCM 1329^T =CGMCC 1.1891^T =ATCC 13752^T.

During a project to investigate bacterial communities, an actinomycete strain, designated CW 108^T, was isolated from a forest soil in Anhui Province, China. It showed high 16S rRNA gene sequence similarity to *Arthrobacter atrocyaneus* DSM 20127^T, but the two strains showed moderate or low 16S rRNA gene sequence similarity (less than 95.2%) to all other members of the related genera *Citricoccus*, *Micrococcus*, *Zhihengliuella*, *Arthrobacter*,

Acaricomes and *Renibacterium* within the family *Micrococcaceae*. Furthermore, strain CW 108^T and *Arthrobacter atrocyaneus* DSM 20127^T displayed well differentiated morphological and physiological characteristics. The two strains could also be separated from other members of the *Micrococcaceae* by colony colour or obvious growth on PYES (Wieser *et al.*, 2002), YDC (Kuhn & Starr, 1960) and TYB (containing 0.3% yeast extract, 0.2% beef extract, 0.6% tryptone, 0.3% NaCl and 0.001% FeCl₃) media at 41 °C. In order to determine the taxonomic and phylogenetic position of the newly isolated bacterium, the two strains were selected for determination of morphological, physiological, biochemical characteristics and chemotaxonomic compositions and their 16S rRNA gene sequences were also analysed. The results indicated that strains CW 108^T and DSM 20127^T represent

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CW 108^T and DSM 20127^T are EU370704 and EU697388, respectively.

Electron micrographs of cells of strain CW 108^T, photographs of colonies of strains CW 108^T and DSM 20127^T, a 16S rRNA gene sequence-based maximum-parsimony tree and a 2D TLC plate of polar lipids of strain CW 108^T are available as supplementary material with the online version of this paper.

a novel genus and that the two strains should be assigned to two separate species of the new genus.

To investigate morphological, biochemical and physiological characteristics, strains were routinely cultivated using TYB and PYES media at 30 °C. Unless otherwise indicated, phenotypic characteristics were studied using standard procedures (Smibert & Krieg, 1994; Zhou *et al.*, 2007). The morphological characteristics of strain CW 108^T were observed by light microscopy (model XTL-3400; Olympus) and transmission electron microscopy (model H-7650; Hitachi) after incubation for 2 days at 30 °C on PYES agar. Electron microscopy preparations were performed as described by Yoon *et al.* (2003). For the various physiological tests, API 20 NE and API 50 CHB test strips (bioMérieux) were applied according to the manufacturer's instructions.

PCR amplification of the 16S rRNA gene was performed as described by Li *et al.* (2007). 16S rRNA gene sequences were aligned manually with reference sequences retrieved from the GenBank database following BLAST searches. Phylogenetic trees were constructed using the software package MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to Kimura's two-parameter model; Kimura, 1980, 1983) and clustering were based on the neighbour-joining and maximum-parsimony methods. Bootstrap analysis was used to evaluate the topology of the neighbour-joining tree by performing 1000 resamplings (Felsenstein, 1985).

Genomic DNA was prepared for determination of the base composition and DNA–DNA hybridization following the procedure of Marmur (1961). The DNA G+C base content was determined by reversed-phase HPLC according to Mesbah *et al.* (1989) using the strain *Escherichia coli* DH5 α as a control. DNA–DNA hybridizations were carried out by applying optical renaturation methods (Jahnke, 1992; Huß *et al.*, 1983; De Ley *et al.*, 1970). DNA–DNA hybridizations were performed on CW 108^T with reference strain DSM 20127^T in five replicates. Procedures for identification of cell-wall amino acids and sugars followed those described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Respiratory quinones were extracted from lyophilized cells and samples were purified and analysed by HPLC using the procedures reported by Hu *et al.* (2001). Cellular fatty acid composition analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). The fatty acid profile of strain CW 108^T contained C_{14:0} (1.3 %), i-C_{15:0} (30.6 %), ai-C_{15:0} (35.9 %), i-C_{16:0} (1.9 %), C_{16:0} (4.3 %), i-C_{17:0} (3.2 %), ai-C_{17:0} (15.4 %), C_{18:0} (2.2 %) and summed feature 3 (C_{16:1} ω 6c and/or C_{16:1} ω 7c; 1.0 %), and that of strain DSM 20127^T contained i-C_{14:0} (1.4 %), i-C_{15:0} (13.9 %), ai-C_{15:0} (36.3 %), i-C_{16:0} (7.2 %), C_{16:0} (1.6 %), i-C_{17:0} (6.0 %), ai-C_{17:0} (30.2 %) and C_{18:0} (1.0 %).

Strains CW 108^T and DSM 20127^T showed good growth on PYES and TYB media and moderate growth was observed on Luria–Bertani agar (Oxoid). Obvious growth occurred even at 42 °C for CW 108^T. Cells of CW 108^T were non-motile, bent rods (0.5–0.9 μ m wide and 1.7–4.5 μ m long) (Supplementary Fig. S1, available in IJSEM Online). The morphological and physiological characteristics described above are significantly different from those of members of the closely related genera *Citricoccus* and *Micrococcus*. Other physiological and biochemical properties are detailed in the species description.

Almost-complete 16S rRNA gene sequences of the two strains (1385 bp for CW 108^T and 1404 bp for DSM 20127^T) were determined. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain CW 108^T clearly belongs to the family *Micrococcaceae* and formed a deeply separated monophyletic cluster with *Arthrobacter atrocyaneus* DSM 20127^T in the phylogenetic trees (Fig. 1 and Supplementary Fig. S2). 16S rRNA gene sequence similarity between strain CW 108^T and DSM 20127^T was 99.4 %. The two strains showed moderate or low 16S rRNA gene sequence similarity to all other described members of the related genera *Citricoccus*, *Micrococcus*, *Zhihengliuella*, *Arthrobacter*, *Acaricomes* and *Renibacterium* (the maximum similarity, of 95.2 %, was between *Arthrobacter atrocyaneus* DSM 20127^T and *Citricoccus alkalitolerans* YIM 70010^T). The two strains shared the same 16S rRNA gene signature nucleotides and most signature nucleotides of the two strains match those of the family *Micrococcaceae* (Stackebrandt *et al.*, 1997), except at positions 640, 839, 847, 1025, 1036 and 1278 (*E. coli* numbering), where the nucleotides U, A, U, U, C, G and U were replaced by G, C, G, C, U, C and C, respectively.

The DNA G+C contents of strains CW 108^T and DSM 20127^T were 71.1 and 70.3 mol%. The respiratory quinone system of the two strains consisted of MK-9(H₂) and MK-8(H₂) (7:1), and strain CW 108^T contained MK-6(H₂) (<4.6 %) as a minor component. The major fatty acids of the two strains were ai-C_{15:0}, i-C_{15:0} and ai-C_{17:0}. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylmonomethylethanolamine (Supplementary Fig. S3). The polar lipid pattern of CW 108^T is significantly simpler than those detected in related genera of the family *Micrococcaceae* (Zhang *et al.*, 2007; Pukall *et al.*, 2006; Wieser *et al.*, 2002; Altenburger *et al.*, 2002; Sanders & Fryer, 1980). Strain CW 108^T lacks diaminopimelic acid in the cell-wall hydrolysate. The amino acids Lys, Ala, Glu and Gly were detected in a molar ratio of 1:3.5:1:0.2 in peptidoglycan of strain CW 108^T. These results suggest that the new isolate showed peptidoglycan type A3 α , which is consistent with the peptidoglycan type reported for strain DSM 20127^T (Koch *et al.*, 1995; Schleifer & Kandler, 1972). The cell-wall sugars for CW 108^T were galactose, mannose and ribose. DNA–DNA relatedness between strain CW 108^T and strain DSM 20127^T is 52.2 %, which confirmed that the two strains should be allocated to two different species of the same

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the positions of strains CW 108^T and DSM 20127^T. Bootstrap values are shown as percentages of 1000 replicates; only values above 50 % are shown. GenBank accession numbers are shown in parentheses. Bar, 1 substitution per 100 nucleotide positions.

genus. Chemotaxonomic characteristics that differentiate the newly proposed genus *Sinomonas* gen. nov. from related genera in the family *Micrococcaceae* are given in Table 1.

Although phylogenetic analysis and signature nucleotides supported the conclusion that the new isolate and strain DSM 20127^T were members of the family *Micrococcaceae*, the two strains showed moderate distinctions of chemotaxonomic features and differentiating physiological characteristics from related genera in their major fatty acids, respiratory quinones, polar lipids, cell-wall sugars and mesophilic growth. On the basis of a comparison of our phylogenetic and chemotaxonomic results together with phenotypic characteristics determined in this study and other references reported for *Arthrobacter atrocyaneus* DSM 20127^T (Yamada & Komagata, 1972; Interschick *et al.*, 1970), a new genus *Sinomonas* gen. nov. is proposed to house strain DSM 20127^T and the new isolate CW 108^T. The signature nucleotide results for strains DSM 20127^T and CW 108^T also supported the placement of the two isolates in a new genus. The name *Sinomonas flava* gen. nov., sp. nov. is proposed for the newly isolated strain CW 108^T, and *Arthrobacter atrocyaneus* DSM 20127^T is reclassified as *Sinomonas atrocyanea* comb. nov.

Description of *Sinomonas* gen. nov.

Sinomonas (Si.no.mo'nas. M.L. n. *Sina* China; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Sinomonas* a monad from China).

Cells strain Gram-positive or variable and are aerobic, mesophilic rods (or show a rod-coccus cycle). Weak catalase activity is present and oxidase activity is absent. The major fatty acids are ai-C_{15:0}, i-C_{15:0} and ai-C_{17:0}. The respiratory quinone system consists of MK-9(H₂) and MK-8(H₂) in a molar ratio of about 7:1. Polar lipids

consist of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol and a minor amount of phosphatidylmonomethylethanolamine. The peptidoglycan type is A3 α and cell-wall sugars are galactose, mannose and ribose. The DNA G+C content is about 71 mol%. The type species is *Sinomonas flava*.

Description of *Sinomonas flava* sp. nov.

Sinomonas flava (fla'va. L. fem. adj. *flava* yellow, referring to the colour of the colonies).

The following properties are displayed in addition to those given in the genus description. Cells strain Gram-positive and are non-motile, bent rods (0.5–0.9 $\times$ 1.7–4.5 μ m). Colonies are circular, convex and pale yellow-coloured after 2 days cultivation at 30–37 °C on TYB, YDC or PYES media. Growth occurs at 15–42 °C (optimum 30–37 °C) and pH 5.0–9.0 (optimum pH 6.0–8.0). No dark-blue pigment is produced on YDC agar at 30 °C (Supplementary Fig. S4). Growth occurs with 0–3 % NaCl, but does not occur with 4 % NaCl. Displays the following results in API 20NE tests: Tween 80 is hydrolysed but casein, starch, and tyrosine are not hydrolysed; urease, β -galactosidase and lipase activities are present; arginine dihydrolase, tryptophan decarboxylase, ornithine decarboxylase and lysine decarboxylase activities are absent; nitrate is reduced and nitrite is not reduced; citrate is not utilized and gelatin is not hydrolysed; H₂S is not produced; the Voges–Proskauer test is positive; indole is not produced. Displays the following results in API 50CHB tests: aesculin, cellobiose, erythritol, D-fructose, D-glucose, glycerol, inositol, 2-ketogluconate, maltose, D-mannose, mannitol, melezitose, methyl β -D-xyloside, D-ribose, sorbitol, sucrose and turanose are utilized as sole carbon sources; N-acetylglucosamine, D-adonitol, amygdalin, D- and L-arabitol, D- and L-arabinose, arbutin, dulcitol, D- and

Table 1. Characteristics that differentiate *Sinomonas* gen. nov. from related genera in the family Micrococcaceae

Data for reference genera are from Li *et al.* (2005) and Altenburger *et al.* (2002) (*Citricoccus*), Liu *et al.* (2000, 2007) and Wieser *et al.* (2002) (*Micrococcus*), Zhang *et al.* (2007) (*Zhihengliuella*), Altenburger *et al.* (2002) (*Arthrobacter*), Pukall *et al.* (2006) (*Acaricomes*) and Sanders & Fryer (1980) (*Renibacterium*). Components in square brackets are present in some members only; a forward slash (/) separates alternative profiles. ND, No data available. *Arthrobacter* I and II refer to two groups of species within the genus first described by Koch *et al.* (1995).

Genus	Menaquinone(s)	Major fatty acids	Polar lipids*	Peptidoglycan type	Cell-wall sugars†	DNA G+C content (mol%)
<i>Sinomonas</i>	MK-9(H ₂), MK-8(H ₂)	ai-C _{15:0} , i-C _{15:0} , ai-C _{17:0}	DPG, PG, PI, PME	A3α	Gal, Man, Rib	71
<i>Citricoccus</i>	MK-9(H ₂), MK-7(H ₂)	ai-C _{15:0} , ai-C _{17:0} , i-C _{16:0} , i-C _{15:0}	DPG, PG, PI, PL, GL	A4α	ND	64–68
<i>Micrococcus</i>	MK-8(H ₂), [MK-8]	ai-C _{15:0} , i-C _{15:0}	DPG, PG, PI, PL, GL	A2α/A4α	Gal/Man	66–76
<i>Zhihengliuella</i>	MK-9, MK-10	ai-C _{15:0} , i-C _{15:0}	DPG, PI, PG, PL, GL	A4α	Tyr, Glc	66.5
<i>Arthrobacter</i> I	MK-9(H ₂)	ai-C _{15:0} , i-C _{17:0} , i-C _{16:0} , i-C _{15:0}	DPG, PG, PI, DMDG	A3α	Gal/(Glc, Rha, Man)	59–70
<i>Arthrobacter</i> II	MK-8, MK-9	ai-C _{15:0} , i-C _{17:0} , i-C _{16:0} , i-C _{15:0}	DPG, PG, DMDG	A4α	Glc, [Gal]	55–66
<i>Acaricomes</i>	MK-10(H ₂)	ai-C _{15:0} , i-C _{17:0}	DPG, PG, PI	A3α	Gal, Glc	57
<i>Renibacterium</i>	MK-9, MK-10	ai-C _{15:0} , i-C _{17:0}	DPG, GL	A3α	Gal, Rha	52–54

*DMDG, Dimannosyldiacylglycerol; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmonomethylethanolamine; PL, unidentified phospholipid.

L-fucose, D-galactose, gentiobiose, gluconate, glycogen, inulin, 5-ketogluconate, D-lactose, D-lyxose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, L-rhamnose, salicin, L-sorbose, starch, D-tagatose, trehalose, xylitol and D- and L-xylose are not utilized. The fatty acid profile contains ai-C_{15:0}, i-C_{15:0}, ai-C_{17:0}, C_{16:0}, i-C_{17:0}, C_{18:0}, i-C_{16:0}, C_{14:0} and summed feature 3 (C_{16:1}ω6c and/or C_{16:1}ω7c).

The type strain is CW 108^T (=LMG 24447^T =KCTC 19388^T =CCTCC AB 207194^T), which was isolated from the surface layer of a forest soil in Anhui Province, China. The DNA G+C content of the type strain is 71.1 mol% (HPLC).

Description of *Sinomonas atrocyanea* (Kuhn and Starr 1960) comb. nov.

Sinomonas atrocyanea [at'ro.cy.a'ne.a. L. fem. adj. *atra* black; N.L. fem. adj. *cyanea* dark blue; N.L. fem. adj. *atrocyanea* (sic) dark blackish blue, referring to the dark-blue pigment produced under certain conditions].

Basonym: *Arthrobacter atrocyaneus* Kuhn and Starr 1960.

Cells show aerobic and mesophilic growth. Gram staining is variable at different growth stages. Morphological characteristics (colony colour, rod-coccus cycle for cell morphology, mobility, etc.) are variable at different growth stages or under different cultural conditions. Colonies are circular, convex and grey-white after cultivation for 2 days at 37 °C on TYB or PYES media. Dark-blue pigment is produced on YDC agar at 30 °C (Supplementary Fig. S4). Displays the following results in API 20NE tests: urease and β-galactosidase are present; arginine dihydrolase, tryptophan decarboxylase, ornithine decarboxylase and lysine decarboxylase activities are absent; nitrate is reduced but nitrite is not reduced; citrate is not utilized and gelatin is not hydrolysed; H₂S is not produced; the Voges-Proskauer test is positive; indole is not produced; glucose, inositol, rhamnose and sucrose are utilized; amygdalin, D- and L-arabinose, mannose, melibiose and sorbitol are not utilized. The fatty acid profile contains ai-C_{15:0}, ai-C_{17:0}, i-C_{15:0}, i-C_{16:0}, i-C_{17:0}, C_{16:0}, i-C_{14:0} and C_{18:0}. Details of other physiological, biochemical and chemotaxonomic properties are as described by Kuhn & Starr (1960) and Interschick *et al.* (1970).

The type strain is DSM 20127^T =JCM 1329^T =CGMCC 1.1891^T =ATCC 13752^T. The DNA G+C content of the type strain is 70.3 mol% (HPLC).

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