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Chryseobacterium chengduensis sp. nov. isolated from the air of captive giant panda enclosures in Chengdu, China*#

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Abstract: A Gram-negative, aerobic, non-motile, rod-shaped bacterial strain, designated 25-1^T, was isolated from the air inside giant panda enclosures at the Chengdu Research Base of Giant Panda Breeding, China. Strain 25-1^T grew optimally at pH 7.0–8.0, at 28–30 °C and in the presence of NaCl concentrations from 0.0% to 0.5%. 16S rRNA gene sequence analysis indicated that strain 25-1^T belongs to the genus *Chryseobacterium* within the family Flavobacteriaceae and is related most closely to *C. carnis* G81^T (96.4% similarity), *C. lathyri* RBA2-6^T (95.8% similarity), and *C. zeae* JM1085^T (95.8% similarity). Its genomic DNA G+C molar composition was 36.2%. The major cellular fatty acids were *iso*-$C_{15:0}$ (44.0%), *iso*-$C_{17:0}$ 3OH (19.8%) and $C_{16:1}$ ω7c/$_{16:1}$ ω6c (12.7%). The only isoprenoid quinone was menaquinone 6 (MK-6). The major polar lipids were phosphatidylethanolamine, two unidentified amino lipids and two unidentified lipids. The DNA–DNA relatedness between strain 25-1^T and *C. lathyri* RBA2-6^T was 38%. Phenotypic, genotypic, and phylogenetic characteristics indicated that strain 25-1^T is a novel member of the genus *Chryseobacterium*, for which the name *C. chengduensis* sp. nov. is proposed. The type strain is 25-1^T (CCTCC AB2015133^T=DSM 100396^T).

Key words: *Chryseobacterium chengduensis*, Giant panda, 16S rRNA sequencing, Phylogenetic analysis, Strain 25-1^T
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1 Introduction

The genus *Chryseobacterium* was first described by Vandamme *et al.* (1994) and assigned to the family Flavobacteriaceae of the phylum Bacteroidetes. Descriptions of novel *Chryseobacterium* species have been growing rapidly in recent years. Currently, the genus contains over 90 species with valid published names (http://www.bacterio.net/chryseobacterium.html). *Chryseobacterium* strains are widely distributed in aquatic and soil environments, plant rhizospheres, sediments, and food sources (Cho *et al.*, 2010; Park *et al.*, 2013; Loch and Faisal, 2014; Kämpfer *et al.*, 2014a; 2014b; 2015a). However, to the best of our knowledge, no airborne representatives of the genus have yet been described. Some *Chryseobacterium* strains are significant as novel sources of bioactive compounds, such as antioxidants, prebiotics, or as sulfobacin and protease producers (Chaudhari *et al.*, 2009; Wang *et al.*, 2011; Kim H.S. *et al.*, 2012). Moreover, some species, including *C. indologenes*, *C. oranimense*, and *C. gleum*, are opportunistic human pathogens implicated in nosocomial infections (Monteen *et al.*, 2013; Lo and Chang, 2014; Nemli *et al.*, 2015; Sharma *et al.*, 2015). In veterinary medicine, no

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relevant *Chryseobacterium* species have been identified as a pathogen of domestic animals; nevertheless, numerous fish-associated species have been isolated from skin and muscle ulcerations, gill hemorrhage and hyperplasia, and from fish showing general signs of septicemia (Ilardi *et al.*, 2009; Loch and Faisal, 2014). Cells are Gram-negative, strictly aerobic, non-motile, non-spore-forming, rods. They are pigmented yellow by flexirubin-type pigments with menaquinone 6 (MK-6) as the predominant respiratory quinone, branched-chain fatty acids (*iso*-$C_{15:0}$, *iso*-$C_{17:0}$ 3-OH, and *iso*-$C_{17:1}$ ω9c) as the major fatty acids and a DNA G+C content in the range from 29% to 39% (Kämpfer *et al.*, 2009; Bernardet *et al.*, 2010; Yang *et al.*, 2015).

In this study, we investigated the cultivable bacterial community in the air of giant panda enclosures at the Chengdu Research Base of Giant Panda Breeding in Sichuan Province, located in southwestern China. Based on differences in colony morphology, 28 pure cultures were selected for 16S rRNA gene sequencing and phylogenetic analysis. Species included members of the genera *Micrococcus*, *Chryseobacterium*, *Leuconostoc*, *Staphylococcus*, *Pseudomonas*, *Kocuria*, *Bacillus*, *Exiguobacterium*, *Acinetobacter*, *Escherichia*, *Rothia*, and *Dietzia*. The strain designated 25-1^T was characterized using a polyphasic taxonomy approach, including evaluation of its morphological, biochemical, and phylogenetic characteristics. Unfortunately, *C. carnis* G81^T and *C. zeae* JM1085^T had not been released from culture collections at the time of these investigations and so were not included as reference strains. Therefore, all tests were performed on the new isolate and on *C. lathyri* RBA2-6^T, which was acquired from the National Institute of Technology and Evaluation (NITE) Biological Resource Center (NBRC). The data obtained revealed that strain 25-1^T should be assigned to the genus *Chryseobacterium* as the type strain of a novel species.

2 Materials and methods

2.1 Culture conditions and phenotypic characteristics

Strain 25-1^T was isolated from the cultivable bacterial community in the air of a giant panda enclosure by exposing a petri dish containing tryptic soy agar (TSA, Difco, Leeuwarden, the Netherlands) medium for 15 min. For further analysis, strain 25-1^T was cultivated on Luria-Bertani (LB) agar (Difco) at 30 °C. The presence of flexirubin type pigments was investigated using a 20% (0.2 g/ml) KOH solution according to the study of Bernardet *et al.* (2002). Gram staining was determined using the non-staining method described by Buck (1982). Cellular morphology, motility, and other physiological characteristics were evaluated as previously described (Wen *et al.*, 2016). Cellular morphology was observed by light microscopy (Olympus; magnification 61 000×) and cell size was determined by transmission electron microscopy (H-600-A2; Hitachi, Tokyo, Japan) using cells from an exponentially growing culture. Motility tests were performed using LB broth with 0.3% (3 g/L) agar. Growth temperatures (4, 10, 15, 20, 25, 28, 30, 37, 40, 45, and 50 °C) and pH (2.0–10.0, at intervals of 1.0 pH unit) were monitored during 7 d of incubation in LB broth as described by Xu and Wu (2005). NaCl tolerance was tested in LN medium (LB without NaCl) supplemented with 0%, 0.5%, 1.0%–5.0% (at intervals of 1%) (1%=0.01 g/ml) NaCl during 7 d of incubation. Anaerobic growth was investigated by incubation in an anaerobic chamber (Mitsubishi Gas Chemical, Tokyo, Japan) at 30 °C for 7 d on LB agar.

2.2 Biochemical characteristics and microbial sensitivity test

A number of key characteristics were tested using conventional procedures, as described by Smibert and Krieg (1994) and Skerman (1967), i.e., the production of catalase, oxidase, hydrogen sulphide and indole, and hydrolysis of Tween 80, starch, and gelatin. Some strain 25-1^T and *C. lathyri* RBA2-6^T biochemical reactions were detected using a bacterial biochemical trace kit (Hangzhou Microbial Reagent Co., Ltd., Hangzhou, China), which included the following substances: β-galactosidase, arginine decarboxylase, ornithine decarboxylase, nitrate reduction, mannose, adipic acid, arabinose, trehalose, cellobiose, lactose, salicin, and acetamide. The additional biochemical and physiological properties of strain 25-1^T and *C. lathyri* RBA2-6^T were determined using the BD Phoenix™-100 automated microbiology system (Becton Dickinson, New Jersey, USA), according to the manufacturer's instructions. The biological principles of the Analytic Products INC (API) and Phoenix systems are similar (Wen *et al.*, 2016), but the Phoenix system is automated and can

handle a higher number of tests. Each negative identification (NID) panel contains two fluorescent positive control wells and 45 substrates (O'Hara, 2006). The sensitivity of strain 25-1^T to various antibiotics was determined as previously described (Wen *et al.*, 2016), by spreading bacterial suspensions on LB agar plates and applying filter paper discs containing the following antibiotics (μg per disc): vancomycin (30), sulfamethoxazole (100), tetracycline (30), lincomycin (10), spectinomycin (100), kanamycin (30), furazolidone (100), streptomycin (10), erythromycin (15), trimesulf (25), ciprofloxacin (5), rifampicin (5), ampicillin (10), florfenicol (30), penicillin (10), gentamicin (10), amikacin (30), cephalothin (30), ceftazidime (30), doxycycline (30), cefotaxime (30), and enrofloxacin (5) (all obtained from Hangzhou Microbial Reagents). The strain was incubated in the presence of the antibiotics at 30 °C for 24 h.

2.3 16S rRNA sequencing and phylogenetic analysis

The 16S rRNA gene of strain 25-1^T was amplified by polymerase chain reaction (PCR) using two universal primers as described previously (Greisen *et al.*, 1994), and the amplification products were sequenced by Invitrogen Biotechnology (Shanghai, China). The sequence was compared with sequences available in the EzTaxon Server (http://eztaxon-e.ezbiocloud.net; Kim O.S. *et al.*, 2012) and GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of strain 25-1^T and the type strains of published *Chryseobacterium* species were aligned using the Clustal_X program (Thompson *et al.*, 1997). Phylogenetic analysis was performed using the MEGA 6.0 offline software (Tamura *et al.*, 2013) and PHYML online web server (Guindon *et al.*, 2005). Phylogenetic trees were constructed using the neighbor-joining, maximum-likelihood (Felsenstein, 1981), and maximum-parsimony (Kluge and Farris, 1969) methods in the MEGA 6.0 program, with bootstrap analysis based on 1000 replicates (Felsenstein, 1985).

2.4 Chemotaxonomic and genomic analyses

The DNA G+C composition of strain 25-1^T was determined using the thermal denaturation method (Mandel and Marmur, 1968) with *Escherichia coli* K-12 as a control. Genomic DNA was extracted and purified using conventional procedures (Sambrook and Russell, 2001). Polar lipids of strain 25-1^T were identified by two-dimensional thin-layer chromatography (TLC) according to the protocols of Tindall (1990). The respiratory quinones of strain 25-1^T were extracted and analyzed by high performance liquid chromatography (HPLC) as described by Xie and Yokota (2003), using *C. takakiae* CGMCC1.13488^T as a reference strain. The whole cell fatty acids of strains 25-1^T and *C. lathyri* RBA2-6^T grown on LB agar at 30 °C for 48 h were analyzed using the Sherlock microbial identification system (MIDI) and identified using the MIDI software package Version 6.0 based on the TSBA6 6.00 database. The four above-mentioned analyses were performed at the China General Microbiological Culture Collection Center (CGMCC). Genomic DNA–DNA hybridizations were carried out between strain 25-1^T and *C. lathyri* RBA2-6^T using the fluorometric method (Ezaki *et al.*, 1989), at the Guangdong Microbiology Culture Center (GIMCC).

3 Results and discussion

3.1 Morphological and physiological characteristics

Cells of strain 25-1^T were observed to be non-motile, strictly aerobic, Gram-stain-negative, and rod-shaped (0.44–0.48 μm wide and 0.88–0.92 μm long; Fig. S1). Colonies on LB agar were non-transparent, yellow-pigmented, circular, and smooth with regular edges after 3 d of incubation at 30 °C. The non-fluorescent and non-diffusible yellow pigments belong to the flexirubin type. Cells grow in LN medium with 0%–2% NaCl with an optimum concentration of 0%–0.5%. Good growth occurs at 28–30 °C and no growth occurs below 10 °C or above 40 °C. Growth occurs at pH 6.0–9.0 and optimally at pH 7.0–8.0. The characteristics of strain 25-1^T and *C. lathyri* RBA2-6^T are shown in Tables 1 and 2. Strain 25-1^T was able to use acetate, adonitol, and D-mannitol, but unable to use ornithine, sorbitol, or sucrose. In contrast to *C. lathyri* RBA2-6^T, strain 25-1^T was negative for L-glutamic acid-AMC, γ-glutamyl-NA, L-proline-NA, bis-*p*NP-phosphate (*p*NP: *p*-nitropheno), *p*NP-β-D-glucoside, and esculin hydrolysis.

3.2 Phylogenetic analysis

The 16S rRNA gene sequence (1401 bp) of strain 25-1^T was obtained (GenBank accession number KP966546). Strain 25-1^T showed less than 96.4%

Table 1 Characteristics of strain 25-1^T and *C. lathyri* RBA2-6^T from conventional test

Characteristics	1	2	Characteristics	1	2	Characteristics	1	2	Characteristics	1	2
Catalase activity	+	+	Arginine decarboxylase	–	+	Nitrate reduction	–	–	Cellobiose	–	–
Oxidase activity	+	+	Ornithine decarboxylase	+	–	Assimilation			Lactose	–	–
Production			Degradation			Mannose	–	+	Salicin	–	–
Indole	–	–	Starch	+	+	Adipic acid	–	–	Acetamide	–	–
H_2S	–	–	Tween 80	+	+	Arabinose	–	–			
β-Galactosidase	–	–	Gelatin	–	+	Trehalose	–	–			

1: strain 25-1^T; 2: strain *C. lathyri* RBA2-6^T; –: negative; +: positive. Data are from this study unless otherwise stated

Table 2 Characteristics of strain 25-1^T and *C. lathyri* RBA2-6^T from Phoenix system

Characteristics	1	2	Characteristics	1	2	Characteristics	1	2
Hydrolysis			Bis-*p*NP-phosphate	–	+	Sucrose	–	–
Arginine-arginine-AMC	+	+	γ-Glutamyl-NA	–	+	Acetate	+	+
Glycine-proline-AMC	+	+	Urea	–	–	Adonitol	+	+
Glycine-AMC	+	+	Esculin	–	+	Citrate	+	+
Glutaryl-glycine-arginine-AMC	+	+	Utilization			Galacturonic acid	–	–
L-Arginine-AMC	+	+	D-Mannitol	+	+	L-Arabinose	–	–
L-Glutamic acid-AMC	–	+	Tiglic acid	+	+	Malonate	+	+
L-Leucine-AMC	+	+	β-Allose	–	–	L-Rhamnose	–	–
L-Phenylalanine-AMC	+	+	β-Gentiobiose	–	–	Methyl-β-glucoside	–	–
L-Proline-AMC	–	–	α-Ketoglutaric acid	+	+	Maltulose	–	–
L-Pyroglutamic acid-AMC	+	–	Dextrose	–	–	*N*-acetyl-galactosamine	–	–
L-Tryptophan-AMC	+	+	D-Fructose	–	–	*N*-acetyl-glucosamine	–	–
Lysine-alanine-AMC	+	+	D-Galactose	–	–	Ornithine	–	–
L-Proline-NA	–	+	D-Gluconic acid	–	–	Resistance		
*p*NP-β-D-glucoside	–	+	D-Melibiose	–	–	Polymyxin B	+	+
4MU-*N*-acetyl-β-D-glucosaminide	+	+	Sorbitol	–	–	Colistin	+	+

1: strain 25-1^T; 2: strain *C. lathyri* RBA2-6^T; AMC: 7-amido-4-methylcoumarin; MU: methylumbelliferyl; *p*NP: *p*-nitropheno; –: negative; +: positive. Data are from this study unless otherwise stated

sequence similarity to the type strains of all recognized species in the genus *Chryseobacterium*. The highest 16S rRNA sequence similarity was found with *C. carnis* NCTC 13525^T (96.4%) (Holmes *et al.*, 2013), followed by *C. lathyri* RBA2-6^T (95.8%), *C. zeae* JM1085^T (95.8%) (Kämpfer *et al.*, 2014b), *C. shigense* GUM kaji^T (95.8%) (Shimomura *et al.*, 2005), *C. gwangjuense* THG A-18^T (95.7%) (Park *et al.*, 2013), and *C. carnipullorum* 9-R23581^T (95.7%) (Charimba *et al.*, 2013), as determined using the EzTaxon server 2.1. Strain 25-1^T was included in a cluster containing the type strains of *C. carnis* and *C. chaponense*, forming a distinct phylogenetic lineage within the genus *Chryseobacterium* in the neighbor-joining phylogenetic tree (Fig. 1). The phylogenetic position was also supported by the maximum-parsimony and maximum-likelihood trees.

3.3 Chemotaxonomic and genomic characteristics

The cellular fatty acids of strain 25-1^T and *C. lathyri* RBA2-6^T analyzed under the same conditions are shown in Table 3. The predominant fatty acids of strain 25-1^T (≥5%) were *iso*-$C_{15:0}$ (44.0%), *iso*-$C_{17:0}$ 3OH (19.8%), summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c, 12.7%) and summed feature 9 (10-methyl $C_{16:0}$ and/or *iso*-$C_{17:1}$ ω9c, 7.8%). Minor amounts of *anteiso*-$C_{15:0}$ (1.4%), *iso*-$C_{16:0}$ (1.8%), *iso*-$C_{15:0}$ 3OH (3.9%), *iso*-$C_{16:0}$ 3OH (2.0%), and $C_{16:0}$ 3OH (1.2%) were also detected. The presences of major fatty acids, namely *iso*-$C_{15:0}$, *iso*-$C_{17:0}$ 3OH and *iso*-$C_{17:1}$ ω9c, are in accordance with the placement of strain 25-1^T in the genus *Chryseobacterium* (Li and Zhu, 2012; Kämpfer *et al.*, 2015b). However, strain 25-1^T could be readily distinguished from *C. lathyri* by the presence

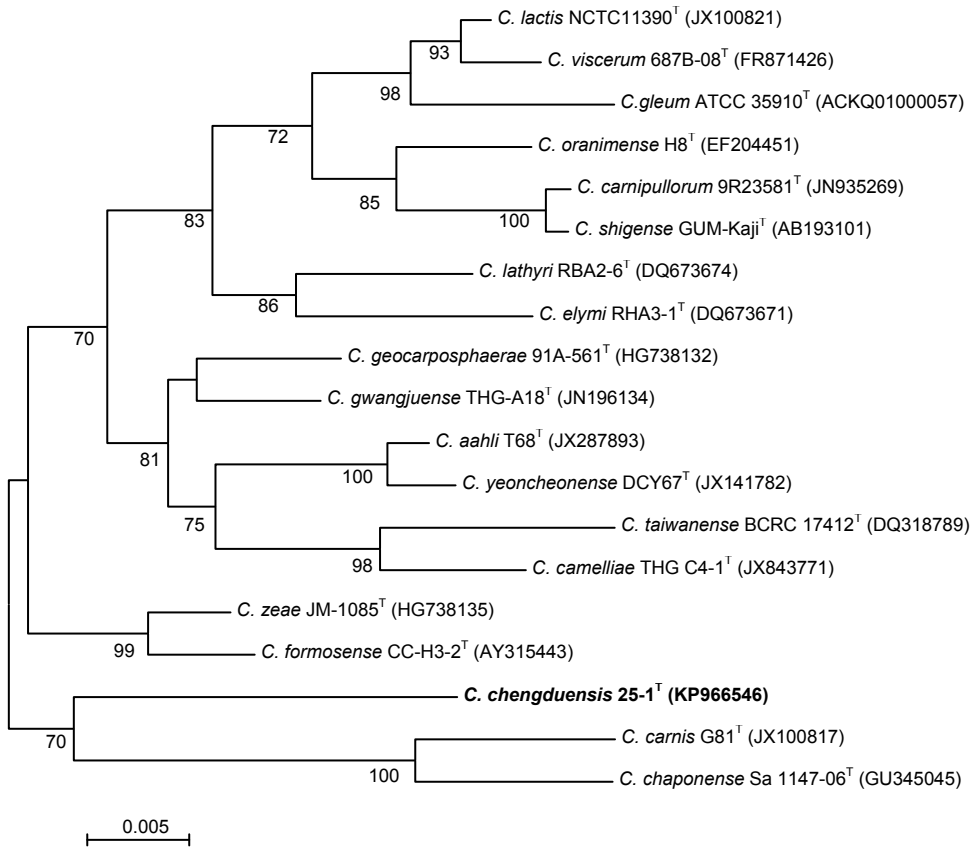

Fig. 1 Phylogenetic tree showing the relationship between strain 25-1^T and the type strains of a selection of recognized *Chryseobacterium* species constructed by the neighbor-joining method based on their 16S rRNA gene sequences
GenBank accession numbers are shown in parentheses. Numbers indicate percentages of occurrence of branch points in 1000 bootstrapped trees. The bar is 0.5% in the legend and 0.005 on the tree

Table 3 Cellular fatty acid content of strain 25-1^T and *C. lathyri* RBA2-6^T

Fatty acid	Content (%)	
	Strain 1	Strain 2
iso-$C_{15:0}$	44.0	53.5
anteiso-$C_{15:0}$	1.4	1.8
iso-$C_{15:1}$ F	ND	1.4
iso-$C_{16:0}$	1.8	TR
$C_{16:0}$	TR	1.8
iso-$C_{15:0}$ 3OH	3.9	5.0
iso-$C_{16:0}$ 3OH	2.0	1.5
$C_{16:0}$ 3OH	1.2	ND
iso-$C_{17:0}$ 3OH	19.8	ND
iso-$C_{17:0}$	ND	1.9
Summed feature 3[a]	12.7	10.7
Summed feature 9[a]	7.8	18.0

Strain 1: *C. chengduensis* sp. nov. 25-1^T; Strain 2: *C. lathyri* RBA2-6^T. All data are from this study. Fatty acids that represented <1.0% of the total in both strains are not shown. ND: not detected; TR: traces (i.e. <1.0%). [a] Summed features are groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c; summed feature 9 consisted of 10-methyl $C_{16:0}$ and/or *iso*-$C_{17:1}$ ω9c

of a high amount of *iso*-$C_{17:0}$ 3OH and a significantly lower amount of summed feature 9. The discrepancies noted in the fatty acid composition of *C. lathyri* RBA2-6^T as determined in this study compared to the data reported in the original description (Cho *et al.*, 2010) may be due to differences in the fatty acid extraction methods, types of gas chromatography, or the culture media used. The isoprenoid quinone of strain 25-1^T was MK-6, which is characteristic of all members of the family Flavobacteriaceae (Kämpfer *et al.*, 2009). The polar lipids of strain 25-1^T were phosphatidylethanolamine (PE), two unidentified amino lipids and two unidentified lipids (Fig. 2), which is in line with other recognized species of the genus *Chryseobacterium* (Kämpfer *et al.*, 2015a). The DNA G+C molar content of strain 25-1^T was 36.2%. This value is within the range reported for *Chryseobacterium* species (Bernardet *et al.*, 2010; Montero-Calasanz *et al.*, 2014). The mean DNA–DNA relatedness between strain 25-1^T and *C. lathyri*

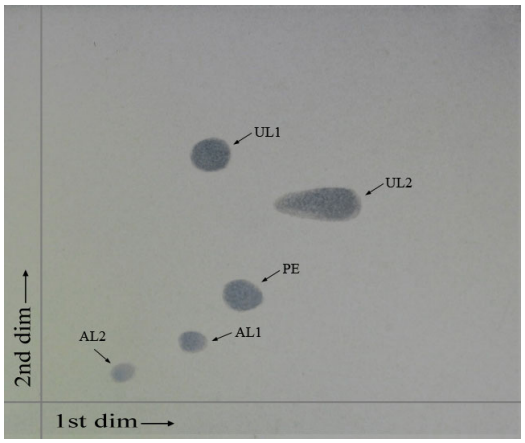

Fig. 2 Total polar lipid analysis of strain *Chryseobacterium chengduensis* 25-1^T carried out by two-dimensional thin-layer chromatography (TLC)

Ascending solvent system: 1st dimension: chloroform/methanol/water (65:25:4, v/v); 2nd dimension: chloroform/methanol/acetic acid/water (80:12:15:4, v/v). Molybdatophosphoric acid was applied for the detection of polar lipids. PE, phosphatidylethanolamine; AL1 and AL2, unidentified amino lipids; UL1 and UL2, unidentified lipids

RBA2-6^T was 38%. This is clearly far below the 70% threshold value that is generally used for prokaryotic species delineation (Stackebrandt *et al.*, 2002).

4 Conclusions

The physiological, chemotaxonomic, and phylogenetic analyses conducted in this study show that strain 25-1^T exhibits the main properties of the genus *Chryseobacterium* but can be differentiated from the closely related type strain, *C. lathyri* RBA2-6^T. The distinctiveness of strain 25-1^T is sufficient to categorize the isolate as a member of a species that is distinguished from the published *Chryseobacterium* species. The low 16S rRNA gene sequence similarity with all other described *Chryseobacterium* species supports the description of strain 25-1^T as a member of a new *Chryseobacterium* species for which we propose the name *C. chengduensis* sp. nov. (*cheng.du.en'sis.* N.L. fem. adj. *chengduensis* pertaining to Chengdu in Sichuan Province, China, where the type strain was isolated). Cells of strain 25-1^T are non-motile, strictly aerobic, Gram-stain-negative, and rod-shaped (0.44–0.48 μm wide and 0.88–0.92 μm long). Colonies on LB agar are non-transparent, yellow-pigmented, circular, and smooth with regular edges after 3 d of incubation at 30 °C. The non-fluorescent and non-diffusible yellow pigments belong to the flexirubin type. Cells grow in LN medium with 0%–2% NaCl with an optimum concentration of 0%–0.5%. Optimal growth occurs at 28–30 °C and no growth occurs below 10 °C or above 40 °C. Growth occurs at pH 6.0–9.0 and optimally at pH 7.0–8.0. Catalase and oxidase are produced, but not indole or H_2S. Starch and Tween 80 are degraded but not gelatin. Nitrate is not reduced to nitrite. Acid is not produced from salicin, acetamide, mannose, β-galactosidase, trehalose, cellobiose, arabinose, and lactose. It is negative for adipic acid and arginine decarboxylase, and positive for the production of ornithine decarboxylase. In the Phoenix system, it is positive for arginine-arginine-AMC, glycine-proline-AMC, glycine-AMC, glutaryl-glycine-arginine-AMC, L-arginine-AMC, L-leucine-AMC, L-phenylalanine-AMC, L-pyroglutamic acid-AMC, L-tryptophan-AMC, lysine-alanine-AMC, acetate, adonitol, citrate, colistin, D-mannitol, α-ketoglutaric acid, malonate, tiglic acid, polymyxin B, and 4MU-*N*-acetyl-β-D-glucosaminide. It is sensitive to vancomycin, tetracycline, rifampicin, ciprofloxacin, florfenicol, doxycycline, amikacin, enrofloxacin, cefotaxime, ceftazidime, furazolidone, and trimesulf, but resistant to kanamycin, spectinomycin, erythromycin, cephalothin, sulfamethoxazole, lincomycin, streptomycin, ampicillin, penicillin, and gentamicin. The major cellular fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{17:0}$ 3OH, summed feature 3 ($C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c) and summed feature 9 (10-methyl $C_{16:0}$ and/or *iso*-$C_{17:1}$ ω9c). The only isoprenoid quinone detected was MK-6. The DNA G+C molar content was 36.2%. The major polar lipids are phosphatidylethanolamine, two unidentified amino lipids and two unidentified lipids.

The type strain, 25-1^T (CCTCC AB2015133^T= DSM 100396^T), was isolated from the air inside giant panda enclosures at the Chengdu Research Base of Giant Panda Breeding in Sichuan Province, China.

Compliance with ethics guidelines

Cai-fang WEN, Li-xin XI, Shan ZHAO, Zhong-xiang HAO, Lu LUO, Hong LIAO, Zhen-rong CHEN, Rong SHE, Guo-quan HAN, San-jie CAO, Rui WU, Qi-gui YAN, and Rong HOU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Fig. S1 Transmission electron micrograph of a negatively stained cell of strain 25-1^T, showing the absence of flagella

中文概要

题　目：分离自成都大熊猫兽舍空气中的一株金黄杆菌属新种的鉴定

目　的：鉴定菌株 25-1^{T}是否是金黄杆菌属的一个新种。

创新点：首次从空气中分离到金黄杆菌属的新种。

方　法：革兰氏染色镜检；磷钨酸染色然后透射电镜观察菌株 25-1^{T}形态结构；全自动生理生化鉴定系统（Phoenix™-100）与传统生理生化反应管相结合；H890 气象色谱仪进行脂肪酸组分分析；薄板双相层析进行极性脂组分分析；反相高压液相色谱分析法进行呼吸琨组分分析；熔解温度法检测 G+C 摩尔含量；16S rRNA 序列测定及系统发生分析。

结　论：根据传统特征分类研究结果（形态特征、培养特性和生理生化特征）、化学分类研究结果（脂肪酸组分、极性脂组分和呼吸琨组分）和遗传特征分类研究结果（G+C 摩尔含量、DNA 同源性测定和 16S rRNA 序列测定及系统发生分析）得出菌株 25-1^{T}为金黄杆菌属的一个新种。

关键词：成都源金黄杆菌；大熊猫；16S rRNA 测序；系统发育树分析；菌株 25-1^T