Description of *Patulibacter americanus* sp. nov., isolated from biological soil crusts, emended description of the genus *Patulibacter* Takahashi *et al.* 2006 and proposal of *Solirubrobacterales* ord. nov. and *Thermoleophilales* ord. nov.

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Two bacterial strains, CP153-3 and CP177-2^T, were isolated from biological soil crusts on the Colorado Plateau, USA, and assigned to the genus *Patulibacter*, according to 16S rRNA gene sequence analysis and phenotypic characteristics. The strains were Gram-positive, aerobic and psychrotolerant and showed positive catalase and negative oxidase reactions. Cells were motile in young cultures, motility consisting of subtle jerking motions, but no flagella could be detected. The strains shared high 16S rRNA gene sequence similarity (99.7 %) and DNA–DNA relatedness (99 %). They contained *meso*-diaminopimelic acid in the cell wall, the major menaquinone was MK-7(H₂) and the predominant fatty acids were C_{18:1}ω9c and iso-C_{15:0}. A low DNA–DNA hybridization value of 20 % with the type strain of the type species of the genus *Patulibacter*, *Patulibacter minatonensis*, as well as phenotypic differences, indicated that the isolates belong to a novel species, for which the name *Patulibacter americanus* sp. nov. is proposed. Strain CP177-2^T (=ATCC BAA-1038^T =DSM 16676^T) was chosen as the type strain. An emended description of the genus *Patulibacter* is also presented. Further, two new orders are created to accommodate several families previously classified in the order *Rubrobacterales* Rainey *et al.* 1997. The first, *Solirubrobacterales* ord. nov., encompasses the families *Solirubrobacteraceae*, *Conexibacteraceae* and *Patulibacteraceae*, and the second, *Thermoleophilales* ord. nov., includes the family *Thermoleophilaceae*.

INTRODUCTION

Biological soil crusts (BSC) are formed by living organisms and their by-products in the top millimetres to centimetres of soil (Belnap, 1993). BSCs prevent soil erosion, fix atmospheric nitrogen and recycle nutrients (Belnap & Gardner, 1993; Belnap, 2002; Johnson *et al.*, 2005). They are particularly common in semiarid and arid environments throughout the world (Belnap, 1994). Culture-independent and -dependent studies on BSCs from the Colorado Plateau of North America have demonstrated the dominance of members of the *Cyanobacteria*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* and low-

G+C Gram-positives in these communities (Reddy & Garcia-Pichel, 2006). Among the isolated bacteria, five novel species from the *Bacteroidetes*, *Alphaproteobacteria* and *Actinobacteria* have been described recently (Reddy & Garcia-Pichel, 2005, 2007; Reddy *et al.*, 2006, 2007). In this work, two novel isolates were subjected to polyphasic characterization, resulting in the proposal of a novel species that belongs to the class *Actinobacteria* (Stackebrandt *et al.*, 1997).

The subclass *Rubrobacteridae*, including the order *Rubrobacterales* and the family *Rubrobacteraceae* with a single genus *Rubrobacter*, was described by Stackebrandt *et al.* (1997) in the work dedicated to a novel classification system for the actinomycete subphylum. The delineation of higher taxa within the established class *Actinobacteria* was proposed solely on the basis of 16S rRNA gene sequence-based phylogenetic clustering and the presence of taxon-specific 16S rRNA gene signature nucleotides. Later, two species of the genus *Thermoleophilum* (Zarilla & Perry, 1984, 1986) were found to form a deep branch within the

Abbreviations: BSC, biological soil crust; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CP153-3 and CP177-2^T are AJ871305 and AJ871306, respectively.

Comparisons of phenotypic, genotypic and chemotaxonomic properties and of fatty acid profiles are available as supplementary material with the online version of this paper.

subclass *Rubrobacteridae* (Yakimov *et al.*, 2003). Further, the genus *Solirubrobacter* (Singleton *et al.*, 2003) was described within *Rubrobacteridae*, and the genus *Conexibacter* (Monciardini *et al.*, 2003) was reported to be another deep-rooted member of this group, with the highest 16S rRNA gene sequence similarity (90%) to *Thermoleophilum*. The above genera were also characterized by significant variations in phenotypic characteristics such as Gram-staining, peptidoglycan composition, the respiratory quinone type and growth temperatures. Owing to the large discrepancy among these genera, Stackebrandt (2004) proposed to classify them into three different families (*Conexibacteraceae*, *Solirubrobacteraceae* and *Thermoleophilaceae*) under the order *Rubrobacterales* and emended the description of the family *Rubrobacteraceae*. The family *Patulibacteraceae* has recently been added to the order (Takahashi *et al.*, 2006).

In the present contribution, besides describing a novel species of *Patulibacter*, we create two new orders, *Solirubrobacterales* ord. nov. and *Thermoleophilales* ord. nov., based on our analyses to denote the high-rank separation between the *Solirubrobacterales*, *Thermoleophilales* and *Rubrobacterales*. Further, the novel species differs from the generic characteristics listed for *Patulibacter* (Takahashi *et al.*, 2006), mainly with respect to the respiratory quinone, and thus an emended description of the genus is proposed.

METHODS

Bacterial strains and growth conditions. Strains CP153-3 and CP177-2^T were isolated from a BSC sample collected from the Colorado Plateau as described previously (Reddy & Garcia-Pichel, 2005). Initially, 0.5 g of crust sample was suspended in Ringer's solution (Reddy *et al.*, 2006) and vortexed for 30 min. The suspension was allowed to settle and then 100 µl supernatant was plated on PGY-BG11 (Reddy & Garcia-Pichel, 2005) and incubated at room temperature for 15 days. Pink-coloured colonies were isolated, purified on 10XPGY-BG11 by streaking and maintained on the same medium.

Morphological, growth and biochemical characteristics. Cell morphology was studied using light microscopy and transmission electron microscopy (TEM). Motility was observed in wet mounts using interference contrast and confirmed by inoculation on 0.4% soft agar medium. For TEM, cells were grown in 10XPGY-BG11 medium up to late-exponential phase, mounted on Formvar-coated copper grids and negatively stained with 0.5% (w/v) uranyl acetate. Grids were examined in TEM mode on a JEOL JEM-1010 electron microscope operated at 60 kV. Growth and biochemical characteristics, carbon assimilation and sensitivity of the cultures to antibiotics were determined as described previously (Reddy & Garcia-Pichel, 2005; Reddy *et al.*, 2006).

Chemotaxonomic characterization. Quantitative analysis of whole-cell fatty acids was performed after growth on tryptic soy agar (Difco) for 2 days at 25 °C. Fatty acid methyl esters were prepared according to the instructions of the Microbial Identification System (MIDI; Microbial ID, Inc.) and analysed using GC/MS. Polar lipids were extracted according to the method of Kates (1972). Approximately 100 mg 10XPGY-BG11-grown cells was suspended

in 1 ml 0.3% saline in a Teflon-lined screw-capped tube. Methanol (10 ml) was added and the mixture was heated to 100 °C for 5 min. After cooling, 5 ml chloroform and 3 ml saline were added and the mixture was vortexed and centrifuged at 3500 g for 10 min to remove the debris. Five millilitres each of chloroform and saline were added to the mixture to separate the phases. The chloroform layer containing polar lipids was collected and dried. Polar lipids were analysed by one-dimensional TLC using a pre-coated silica gel plate, as described previously (Suresh *et al.*, 2004). Cells of CP153-3 and CP177-2^T grown on 10XPGY-BG11 agar were scrapped off and suspended in chloroform/methanol (2:1); the suspension was vortexed and pigments were extracted. After centrifugation at 3500 g for 5 min and evaporation of the chloroform/methanol layer, a UV-visible spectrum was recorded (200–1200 nm) in methanol. Peptidoglycan was prepared as described by Komagata & Suzuki (1987). Qualitative analysis of peptidoglycan amino acids was performed by TLC using the solvent system n-butanol/acetic acid/water (3:1:1 by vol.) and developed with ninhydrin reagent as described by Steiner *et al.* (1998). For quantitative analysis, peptidoglycan amino acids were analysed by the OPA (*o*-phthalaldehyde) method (Babu *et al.*, 2002). Approximately 7 µl hydrolysed peptidoglycan in borate buffer was mixed with 1 µl OPA reagent (74.6 mM OPA and 94.2 mM mercaptopropionic acid prepared in methanol and 0.4 M sodium borate, pH 10.5) in the injection loop and immediately separated on a C18 reversed-phase HPLC column. The C18 reversed-phase column was a Hypersil AA-ODS column from Agilent (2.1 × 200 mm), operated at a flow rate of 0.45 ml min⁻¹, using a linear gradient of 100% A (at *t*=0) to 40% A over 17 min. The percentage of solvent A was then decreased to 0% at *t*=18 min. The composition of solvent A was 20 mM sodium acetate with 0.018% (v/v) triethylamine (pH 7.2) and 3% tetrahydrofuran and solvent B was 20 mM sodium acetate (pH 7.2) with 40% each of acetonitrile and methanol. Eluted amino acids were detected by a fluorescence detector set at excitation and emission wavelengths of 340 and 450 nm, respectively. Isoprenoid quinones were extracted according to the method of Collins *et al.* (1977), separated on HPLC using an isocratic solvent system [methanol/isopropyl ether (3:1, v/v)] and identified by MS (Tamaoka *et al.*, 1983; Tamaoka, 1986).

G+C content, DNA–DNA hybridization and 16S rRNA gene sequencing. Isolation of DNA and determination of the G+C content were carried out as described by Marmur (1961). A mean of two independent experiments is given. The variation between the experiments was less than 2%. DNA–DNA hybridization was carried out spectroscopically as described by De Ley *et al.* (1970). For 16S rRNA gene sequencing, DNA was prepared using the MoBio microbial DNA isolation kit (MoBio Laboratories Inc.) and sequenced as described previously (Reddy & Garcia-Pichel, 2005; Reddy *et al.*, 2000).

Phylogenetic analyses. The 16S rRNA gene sequences obtained for CP153-3 and CP177-2^T were aligned with closely related sequences belonging to the class *Actinobacteria* using CLUSTAL W (Thompson *et al.*, 1994). Pairwise evolutionary distances were computed using the DNADIST program with Kimura's two-parameter model (Kimura, 1980). Phylogenetic trees were constructed using four tree-making algorithms, neighbour-joining, minimum-evolution, the unweighted pair group method with arithmetic means (UPGMA) and maximum-parsimony, using the MEGA 3 software package (Kumar *et al.*, 2004). Bootstrap analysis was performed employing 1000 replicate datasets.

RESULTS AND DISCUSSION

Strains CP153-3 and CP177-2^T formed entire, pink, convex colonies. Colonies of CP177-2^T were slightly mucoid

compared with those of CP153-3. Cells of CP153-3 and CP177-2^T consisted of short rods (bacilli to coccobacilli) that often occurred in pairs (Fig. 1). Under most conditions, cells were non-motile, but very young cultures displayed a subtle cellular jerking. While this jerking was by no means typical bacterial swimming with obvious lateral displacement, soft-agar plates confirmed that those cultures were indeed motile. No flagella were detected on negative-staining TEM inspection. Details of growth, phenotypic and chemotaxonomic characteristics of strain CP177-2^T are reported in the species description. Partial 16S rRNA gene sequences of 1454 nucleotides were obtained for both CP153-3 and CP177-2^T. The strains shared 99.7 % 16S rRNA gene sequence similarity and 99 % DNA–DNA hybridization, indicating that the two isolates are strains of a single species; therefore, only CP177-2^T was characterized in detail.

The taxa with the most similar 16S rRNA gene sequences to these strains were *Patulibacter minatonensis* (Takahashi *et al.*, 2006), *Conexibacter woesei* (Monciardini *et al.*, 2003) and *Solirubrobacter pauli* (Singleton *et al.*, 2003), the type strains of which shared sequence similarities of 98.7, 93.4 and 91.7 %, respectively. These taxa are members of different families, *Patulibacteraceae* (Takahashi *et al.*, 2006), *Conexibacteraceae* and *Solirubrobacteraceae* (Stackebrandt, 2004), respectively. Detailed examination of the 16S rRNA gene sequences of strains CP153-3 and CP177-2^T was consistent with their assignment within the class *Actinobacteria*, since they possessed, for instance, the nucleotide A at position 906 (Stackebrandt *et al.*, 1997), a signature nucleotide of this class (Table 1). The high sequence similarity with the 16S rRNA gene from *P. minatonensis* KV-614^T and the fact that the two novel strains and *P. minatonensis* form a statistically robust, separate clade in various phylogenetic reconstruction

methods (Fig. 2) supported their assignment to the genus *Patulibacter*. DNA–DNA relatedness of only 20 % with the type strain of *Patulibacter minatonensis*, JCM 12834^T, fell short of the suggested 70 % specific threshold, indicating that the novel strains deserve separate species status (Wayne *et al.*, 1987). The species status of CP177-2^T was further supported by the phenotypic differences from *P. minatonensis* (Table 2). For instance, cells of CP177-2^T had variable, jerking motility and colonies were pink, whereas cells of *P. minatonensis* were motile by means of flagella and colonies were pale yellow. Most importantly, the respiratory quinone present in CP177-2^T was MK-7(H₂) and that of *P. minatonensis* is DMK-7, a demethylmenaquinone, which is found in only a few genera (Takahashi *et al.*, 2006). Other differences involving growth, biochemical characteristics, sensitivity to antibiotics and utilization of carbon compounds were also apparent (Table 2).

Taxonomic interpretation of the families *Solirubrobacteraceae*, *Patulibacteraceae* and *Conexibacteraceae*

The members of families *Solirubrobacteraceae*, *Patulibacteraceae* and *Conexibacteraceae* are psychrotolerant to mesophilic and share traits such as the presence of *meso*-diaminopimelic acid in the cell-wall peptidoglycan, C_{18:1}ω9c as the major fatty acid (iso-C_{16:0} and C_{18:1}ω9c in the case of *Solirubrobacteraceae*) and a G + C content above 70 mol%, justifying their inclusion in a single suprageneric taxon, as suggested by the phylogenetic reconstructions. These families differ from members of the *Rubrobacteraceae* and *Thermoleophilaceae* with respect to G + C content, peptidoglycan composition and isoprenoid quinones (Supplementary Table S1, available in IJSEM Online); other differences are listed in Supplementary Tables S1 and S2. Members of these three families form a well-defined phylogenetic clade, with a well-supported separation from the rest of *Rubrobacterales* Rainey *et al.* 1997 in all methods tested, UPGMA, neighbour-joining (Fig. 2), maximum-parsimony and minimum-evolution. Signature nucleotides of *Patulibacteraceae* (Takahashi *et al.*, 2006), *Conexibacteraceae* and *Solirubrobacteraceae* (Stackebrandt, 2004) differ from those of *Rubrobacteraceae* Rainey *et al.* 1997 emend. Stackebrandt 2004 in having G–C, U–A, U–A, G–C, G–C, G–C and U–A at positions 63:104, 657:749, 681:709, 953:1228, 954:1226, 1051:1207 and 1118:1155 (seven signatures), respectively (Table 1). They also differ from members of the *Thermoleophilaceae* at positions 370:391, 580:776, 670:736, 681:709, 941:1342, 1118:1155 and 1311:1326 (seven signatures) (Table 1).

The above characteristics strongly justify the creation of a new order, *Solirubrobacterales* ord. nov., to accommodate the families *Patulibacteraceae* (Takahashi *et al.*, 2006), *Conexibacteraceae* and *Solirubrobacteraceae* (Stackebrandt, 2004). Therefore, we propose the novel order *Solirubrobacterales* ord. nov., named after its oldest

Fig. 1. Cells of strain CP177-2^T freshly grown in 10XPGY-BG11 medium for 2 days, negatively stained with uranyl acetate and examined in TEM. Bar, 0.5 μm.

Table 1. Signature nucleotides of the members of the orders *Solirubrobacterales* ord. nov., *Thermoleophilales* ord. nov. and *Rubrobacterales* Rainey *et al.* 1997 emend. Reddy and Garcia-Pichel

Adapted from Stackebrandt *et al.* (1997), Stackebrandt (2004) and Takahashi *et al.* (2006). Letters in bold indicate signatures that are characteristic of the order.

Position(s)	<i>Solirubrobacterales</i> ord. nov.			<i>Rubrobacterales</i>	<i>Thermoleophilales</i> ord. nov.
	<i>Patulibacteraceae</i>	<i>Conexibacteraceae</i>	<i>Solirubrobacteraceae</i>	<i>Rubrobacteraceae</i>	<i>Thermoleophilaceae</i>
52:359	C-G	U-A	C-G	G-C	C-G
63:104	G-C	G-C	G-C	C-G	G-C
70:98	G-C	G-C	G-C	A-U	G-C
127:234	G-C	G-C	G-C	G-C	G-C
139:224	G-C	G-C	A-U	U-A	G-C
144:178	C-G	U-A	C-G	G-C	C-G
291:309	U-A	U-A	U-A	U-A	U-A
370:391	C-G	C-G	C-G	C-G	G-C
408:434	G-C	A-U	G-C	G-C	G-C
580:776	U-A	U-A	U-A	U-A	C-G
590:649	U-A	U-A	C-G	C-G	C-G
600:638	U-G	U-G	C-G	U-G	C-G
657:749	U-A	U-A	U-A	G-C	U-A
670:736	A-U	A-U	A-U	A-U	G-C
681:709	U-A	U-A	U-A	C-G	C-G
823:877	A-U	G-C	G-C	G-C	G-C
906	A	A	A	A	A
941:1342	A-U	A-U	A-U	A-U	G-C
953:1228	G-C	G-C	G-C	U-A	G-C
954:1226	G-C	G-C	G-C	C-G	G-C
955:1225	U-A	U-A	U-A	U-A	U-A
999:1041	U-A	G-U	U-A	U-A	A-U
1051:1207	G-C	G-C	G-C	C-G	G-C
1115:1185	C-G	C-G	C-G	C-G	C-G
1118:1155	U-A	U-A	U-A	C-G	C-G
1311:1326	A-U	A-U	A-U	A-U	G-C
1410:1490	A-U	U-A	U-A	U-A	U-A

standing genus. The creation of the *Solirubrobacterales* is further justified by the long evolutionary distances observed between the species of *Solirubrobacterales* and *Rubrobacterales* Rainey *et al.* 1997 emend. Reddy and Garcia-Pichel (84.5–92% 16S rRNA gene sequence similarity), which are comparable to the distances that separate other orders, such as the *Actinomycetales* and *Bifidobacteriales* (81–89%).

Taxonomic interpretation of the genus *Thermoleophilum*

The members of the genus *Thermoleophilum* are non-motile rods and stain Gram-negative (although TEM displayed a series of layers characteristic of Gram-positive bacteria; Yakimov *et al.*, 2003), and colonies are non-pigmented. They are thermophilic and contain meso-diaminopimelic acid in the peptidoglycan (Supplementary Table S1). Presently, the genus has two species, *Thermoleophilum album* (Zarilla & Perry, 1984) and *Thermoleophilum minutum* (Zarilla & Perry, 1986;

Yakimov *et al.*, 2003). An earlier comparative analysis based on 16S rRNA gene sequences placed the genus under group 2 of the subclass *Rubrobacteridae* (Yakimov *et al.*, 2003). More recently, Stackebrandt (2004) created the family *Thermoleophilaceae* to accommodate the two species within the order *Rubrobacterales*. In the present analyses, all of the phylogenetic algorithms indicated that the genus *Thermoleophilum* formed a deeply branched, robust cluster of its own (Fig. 2) and a sister rather than a subordinate taxon to the *Rubrobacterales* Rainey *et al.* 1997. Further, they showed eight signature nucleotides that differed from *Solirubrobacterales* ord. nov. and 15 signatures at positions 52:359, 63:104, 139:224, 144:178, 370:391, 580:776, 600:638, 657:749, 670:736, 941:1342, 953:1228, 954:1226, 999:1041, 1051:1207 and 1311:1326 that differed from the rest of *Rubrobacterales* Rainey *et al.* 1997 emend. Reddy and Garcia-Pichel (Table 1). Thus, the differences in signature nucleotides and the phylogenetic reconstructions clearly support the creation of a higher taxonomic rank for this family, for which the name *Thermoleophilales* ord. nov. is proposed. Although not

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain CP177-2^T and related reference micro-organisms of the class *Actinobacteria*. Bootstrap values (expressed as percentages of 1000 replications) greater than 40 % are indicated at nodes. Some clades have been collapsed into triangles for clarity, with the number of sequences used in each indicated in parentheses. Bar, 0.02 substitutions per nucleotide position.

much information is available about the phenotypic characteristics of the species of *Thermoleophilum*, traits such as their thermophilic nature, Gram-negative staining reaction (although electron microscopy of the cell wall showed morphology typical of Gram-positive bacteria) and lack of pigmentation support the delineation of *Thermoleophilales* ord. nov. from *Solirubrobacterales* ord. nov. (Supplementary Table S1). Members of the order *Thermoleophilales* ord. nov. differ from members of *Rubrobacterales* Rainey *et al.*, 1997 emend. Reddy and Garcia-Pichel with respect to pigmentation, Gram-staining and peptidoglycan (Supplementary Table S1).

Emended description of the order *Rubrobacterales* Rainey *et al.* 1997

Contains the family *Rubrobacteraceae*. Known members are thermophilic, non-motile rods–cocci that stain Gram-positive and contain L-Lys as the diamino acid at position 3 of the peptidoglycan and MK-8 as the predominant menaquinone. Further characterized by the presence of 16S rRNA gene sequence signatures C–G, A–U, U–A, G–C, G–C, U–A, C–G and C–G at positions 63:104, 70:98, 139:224, 144:178, 657:749, 953:1228, 954:1226 and 1051:1207, respectively. The type genus is *Rubrobacter*.

Description of *Solirubrobacterales* ord. nov.

Solirubrobacterales (So'li.ru.bro.bac'te.ra'les. N.L. masc. n. *Solirubrobacter* type genus of the order; suff. -ales ending denoting an order; N.L. fem. pl. n. *Solirubrobacterales* the *Solirubrobacter* order).

Contains the families *Solirubrobacteraceae*, *Conexibacteraceae* and *Patulibacteraceae*. Members of the order are Gram-positive, mesophilic, sometimes psychrotolerant and contain *meso*-diaminopimelic acid as the diamino acid at position 3 of the peptidoglycan. Further characterized by the presence of 16S rRNA gene sequence signature nucleotides G–C, G–C, C–G, U–A, U–A, A–U, U–A, A–U, G–C, G–C, G–C, U–A and A–U at positions 63:104, 70:98, 370:391, 580:776, 657:749, 670:736, 681:709, 941:1342, 953:1228, 954:1226, 1051:1207, 1118:1155 and 1311:1326, respectively. Differ from the *Rubrobacterales* Rainey *et al.* 1997 emend. Reddy and Garcia-Pichel in containing U–A at positions 681:709 and 1118:1155. Other differences in signature nucleotides from *Rubrobacterales* Rainey *et al.* 1997 (Stackebrandt, 2004) and *Thermoleophilales* ord. nov. are listed in Table 1. The type genus is *Solirubrobacter*.

Description of *Thermoleophilales* ord. nov.

Thermoleophilales (Ther.mo'le.o.phi.la'les. N.L. neut. n. *Thermoleophilum* type genus of the order; suff. -ales ending denoting an order; N.L. fem. pl. n. *Thermoleophilales* the *Thermoleophilum* order).

Contains the family *Thermoleophilaceae*. Members stain Gram-negative (TEM demonstrates a series of layers typical of Gram-positive bacteria), are thermophilic and contain *meso*-diaminopimelic acid as the diamino acid in the peptidoglycan. The order is further characterized by the presence of 16S rRNA gene sequence signature nucleotides at various positions as listed in Table 1. *Thermoleophilum* is the type genus.

Emended description of the genus *Patulibacter* Takahashi *et al.* 2006

Patulibacter (Pa.tu'li.bac'ter. L. adj. *patulus* spreading; N.L. masc. n. *bacter* from Gr. n. *baktron* a rod; N.L. masc. n. *Patulibacter* rod with spreading growth).

Cells are Gram-positive, non-endospore-forming, rod-shaped, aerobic, catalase-positive and oxidase-negative; motility is variable. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid as diamino acid along with alanine and glutamic acid. The fatty acid profile is dominated by anteiso-C_{15:0}, anteiso-C_{17:0} and C_{18:1ω9c}. Mycolic acids are absent. The predominant isoprenoid quinone is DMK-7 or MK-7(H₂). The DNA G + C content is approximately 72 mol%. The type species is *Patulibacter minatonensis*.

Description of *Patulibacter americanus* sp. nov.

Patulibacter americanus (a.me.ri.ca'nus. N.L. masc. adj. *americanus* American, referring to the isolation of the type strain from the Colorado Plateau).

Colonies are pink-pigmented, convex, entire, smooth and slightly mucoid. Cells are short rods that stain Gram-

Table 2. Comparison of phenotypic characteristics of strains CP177-2^T and CP153-3 and *P. minatonensis* JCM 12834^T

Data for *P. minatonensis* JCM 12834^T were taken from Takahashi *et al.* (2006). –, Negative; +, positive; ND, no data available; S, sensitive; R, resistant; MK, menaquinone; DMK, demethylmenaquinone.

Characteristic	CP153-3	CP177-2 ^T	<i>P. minatonensis</i> JCM 12834 ^T
Colony colour	Pink	Pink	Pale-yellow
Motility	Variable (jerking)	Variable (jerking)	Motile by flagella
Growth temperature range (°C)	5–30	10–30	16–28
Lipase	+	+	–
Phosphatase	+	–	+
Sensitivity to antibiotics (µg per disc)			
Aztreonam (30)	S	R	R
Chloramphenicol (30)	R	S	S
Cephalothin (30)	R	S	ND
Erythromycin (2)	S	R	S
Sulfisoxazole (300)	R	S	ND
Tetracycline (30)	R	S	S
Utilization of carbon compounds			
Arabinose	–	–	+
Cellobiose	–	–	+
Glucose	–	+	ND
Galactose	–	–	+
Inositol	–	–	+
Maltose	–	+	+
Mannitol	–	–	+
Mannose	–	–	+
Rhamnose	–	–	+
Ribose	–	–	+
Sucrose	+	+	–
Sorbitol	–	+	–
Xylose	–	+	+
Arginine	–	+	ND
Serine	–	–	+
Menaquinone	MK-7(H ₂)	MK-7(H ₂)	DMK-7

positive. Motility is variable and, when present, occurs through jerking cellular motions. Growth is observed at 10–30 °C (but not at 37 °C), with an optimum at 25 °C, and at pH 5–9, with an optimum at pH 7. Tolerates NaCl concentrations of less than 3 %. Tests positive for catalase and lipase but negative for oxidase, urease, β -galactosidase, gelatinase, phenylalanine deaminase and arginine, lysine and ornithine decarboxylases. Also negative for the methyl red test, Voges–Proskauer reaction, indole production and growth on Simmons' citrate. Does not hydrolyse casein, cellulose, aesculin or starch and can not produce H₂S or reduce nitrate to nitrite. Utilizes D-glucose, inulin, D-laevalose, melibiose and sucrose as sole carbon sources. Can not grow on acetate, citrate, dextran, ethanolamine, D-fructose, glycerol, pyruvate, succinate, L-alanine, L-aspartic acid, L-cysteine, L-glycine, L-glutamine, L-glutamic acid, L-histidine, L-leucine, L-lysine, L-phenylalanine, L-tryptophan, L-tyrosine, adenine, cytosine, guanine, thymidine, nicotinic acid, tartaric acid, adonitol, dulcitol, fumaric acid, lactose, lactic acid, raffinose, L-sorbose, trehalose, L-asparagine, L-isoleucine, L-methionine, L-proline, L-threonine, L-valine, oxalate, phenanthrene or indole. Sensitive to

(µg per disc unless indicated) bacitracin (10 U), ceftriaxone (30), doxycycline (30), gentamicin (10), novobiocin (30), polymyxin B (300 U), rifampicin (30), streptomycin (10) and vancomycin (30) and resistant to azithromycin (50), carbenicillin (100), ciprofloxacin (5), colistin (10), ethambutol (50), nitrofurantoin (150), penicillin (10 U), sulfathiazole (300) and trimethoprim (5). Additional characteristics are listed in Table 2. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diamino acid with alanine and glutamic acid in the ratio of 1:2:1 and MK-7(H₂) as the sole respiratory quinone. Methanol extracts exhibit absorption maxima at 327, 395, 434, 459 and 488 nm. Fatty acids are listed in Supplementary Table S2. Polar lipids include unknown phospholipid 1, unknown phospholipid 2, which co-migrated with phosphatidylinositol, and phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol (cardiolipin). The G + C content of the DNA of the type strain is 72 mol%.

The type strain is CP177-2^T (=ATCC BAA-1038^T =DSM 16766^T), isolated from BSC from the Colorado Plateau, USA. Strain CP153-3 (=ATCC BAA-1037), isolated from

the same source, is a reference strain. Phenotypic characteristics of the type strain are the same as those described above for the species; differences between strains CP177-2^T and CP153-3 are listed in Table 2.

Note added in preparation

While this article was under review, another species of the genus *Solirubrobacter*, *Solirubrobacter soli*, was described (Kim *et al.*, 2007).

ACKNOWLEDGEMENTS

The research was funded by the National Science Foundation Biotic Surveys and inventories grant 0206711 to F.G.P. The authors also thank the editor for her keen reviewing of our paper and also editing part of the text.

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