Belnapia moabensis gen. nov., sp. nov., an alphaproteobacterium from biological soil crusts in the Colorado Plateau, USA

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Strain CP2C^T was isolated from biological soil crusts in the Colorado Plateau, USA. The isolate was aerobic, facultatively fermentative, Gram-negative, non-motile and red-pigmented (due to the presence of carotenoids), but did not contain bacteriochlorophyll *a*. The strain tested positive for catalase, oxidase and urease and was negative for lysine and ornithine decarboxylases and arginine dihydrolase. The major fatty acids present were C_{18:1}ω7c and C_{16:0}. It had a high DNA G + C content of 75 mol%. Comparisons of 16S rRNA gene sequences identified bacteriochlorophyll *a*-producing strains of *Paracraurococcus ruber* (94.9%), *Craurococcus roseus* (92.2%) and *Roseococcus thiosulfatophilus* (92.3%), as well as non-bacteriochlorophyll *a*-producing bacteria *Muricoccus roseus* (94.9%), *Roseomonas gilardii* (94.2%) and *Roseomonas mucosa* (93.8%), as the bacteria most closely related to strain CP2C^T. Phylogenetically, CP2C^T was placed roughly equidistantly from the above organisms. Based on its phylogenetic placement and morphological and physiological characteristics, strain CP2C^T is assigned to a new genus in the α-1 subgroup of the *Proteobacteria*, for which the name *Belnapia* gen. nov. is proposed. Strain CP2C^T (= ATCC BAA-1043^T = DSM 16746^T) is proposed as the type strain of the type species of this genus, with the name *Belnapia moabensis* gen. nov., sp. nov.

Biological soil crusts (BSCs) are notoriously slow-growing microbial communities formed by living organisms and their by-products in the top centimetres of the soil, creating a crust of soil particles bound together by organic materials (Belnap, 1993). BSCs are thought to contribute to a number of processes in the environment, especially to those that occur at the land surface or soil–air interface. These include soil stability, erosion resistance, atmospheric N₂ fixation, nutrient contributions to plants, soil–plant–water relations, infiltration, seedling germination and plant growth (Belnap & Gardner, 1993; Belnap, 2002; Johnson *et al.*, 2005). They are particularly common in semiarid and arid environments throughout the world (Belnap, 1994). BSCs are dominated by cyanobacteria, lichens and sometimes by mosses or eukaryotic algae as primary producers (Belnap *et al.*, 2001).

Abbreviations: Bchl *a*, bacteriochlorophyll *a*; BSC, biological soil crust; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CP2C^T is AJ871428.

A 16S rRNA gene sequence similarity matrix, a comparison of nucleotides in the 16S rRNA gene sequence between *Belnapia moabensis* and related species and UPGMA and maximum-parsimony phylogenetic trees are available as supplementary material in IJSEM Online.

Bacteria and fungi are the major heterotrophic components, but little is known about their taxonomic placement. Recent survey studies from various geographical settings (L. M. Nagy, P. Alejandro and F. Garcia-Pichel, unpublished; G. S. N. Reddy and F. Garcia-Pichel, unpublished; Smith *et al.*, 2004) point to the dominance of poorly known and probably novel taxa of *Actinobacteria*, *Proteobacteria*, ‘*Bacteroidetes*’ and low-DNA G + C-content Gram-positive bacteria. Attempts at cultivation and characterization of these bacteria are limited to *Dyadobacter crusticola*, belonging to the ‘*Bacteroidetes*’ (Reddy & Garcia-Pichel, 2005). In the present study, we characterized a BSC isolate belonging to the *Alphaproteobacteria* using polyphasic taxonomic methods. The phenotypic, chemotaxonomic and phylogenetic analyses established that the isolate does not belong to any of the genera described so far. Therefore, it was classified under a new genus, *Belnapia* gen. nov., with the name *Belnapia moabensis* sp. nov.

Strain CP2C^T was isolated from a BSC sample near the town of Moab (UT, USA), in the Colorado Plateau (38° 34′ 984″ N and 109° 31′ 451″ W; collected between 20 and 22 May 2003). The physico-chemical characteristics of the sampling site have been described by Garcia-Pichel *et al.* (2003) and Johnson *et al.* (2005). Initially, a 0.5 g sample was suspended

in Ringer's solution (9 g NaCl, 0.042 g KCl, 0.025 g CaCl₂ and 100 ml distilled water) and shaken for 30 min. The suspension was allowed to settle and 100 µl supernatant was plated on PGY-BG11 agar (Reddy & Garcia-Pichel, 2005) and incubated in the dark at room temperature. A red-pigmented colony was isolated, purified and maintained on PGY-BG11.

Cell morphology was studied using light microscopy and scanning electron microscopy (SEM). For SEM, cells were mounted on Formvar-coated copper grids and negatively stained with 0.5% (w/v) uranyl acetate. Grids were examined in a JEM-1010 SEM (JEOL) operated at 60 kV. Growth at different temperatures was determined using 10×PGY-BG11 medium. For growth at different pH, the components of 10×PGY-BG11 were dissolved in 0.1 M phosphate buffer with pH values ranging from 5 to 12. Tolerance to salt was observed by the addition of NaCl (0.5–6%, final concentration) in 10×PGY-BG11. For all biochemical tests, cells were grown on 10×PGY-BG11. Catalase activity was determined by bubble production in 30% (v/v) aqueous hydrogen peroxide solution. Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck) to a deep purple or blue colour. Nitrate reduction, hydrolysis of aesculin, casein, starch, Tween 80, urea and gelatin and various enzyme activities were determined on 10×PGY-BG11 according to previously described methods (Lanyi, 1987; Smibert & Krieg, 1994). Carbon assimilation tests were performed by adding each carbon compound, at a final concentration of 0.5%, to a base of BG11 medium lacking citric acid (Reddy & Garcia-Pichel, 2005). Antibiotic susceptibility was assayed with antibiotic discs, according to the Kirby–Bauer method (Becton Dickinson Microbiological systems). For testing aerobic and anaerobic photoautotrophic growth, culture was inoculated into liquid and streaked onto agarized BS-V (Saitoh & Nishimura, 1996) or PNP media at pH 6.8 and incubated in the light at room temperature. PNP contained, (l⁻¹), 0.5 g NaCl, 0.4 g MgCl₂·6H₂O, 0.1 g CaCl₂·2H₂O, 0.2 g KH₂PO₄, 0.05 g KCl, 0.5 g Na₂HCO₃, 2.38 g HEPES, 1 ml 1000× vitamin solution and 1 ml trace element solution. The vitamin solution contained, (100 ml⁻¹); 5 mg biotin, 100 mg thiamin, 100 mg nicotinic acid, 50 mg *p*-aminobenzoic acid, 1 mg vitamin B₁₂, 50 mg calcium pantothenate, 50 mg pyridoxine hydrochloride, 50 mg folic acid and 20 mg trisodium EDTA. The composition of the trace element solution used was as follows, (l⁻¹), 10 mg ZnSO₄·7H₂O, 3 mg MnCl₂·4H₂O, 30 mg H₃BO₃, 20 mg CoCl₂·6H₂O, 1 mg CuCl₂·2H₂O, 2 mg NiCl₂·6H₂O and 3 mg Na₂MoO₄. Anaerobic conditions were achieved in GasPak jars with GasPak Plus anaerobic system envelopes (Becton Dickinson). The head space of the jars contained CO₂ and H₂. The ability of the culture to fix nitrogen was tested using BG11 medium without nitrate (BG11⁰), but containing 0.5% glucose (w/v) as the carbon source.

Quantitative analysis of whole-cell fatty acids was performed by growing strain CP2C^T on tryptic soy agar medium at

25 °C. Fatty acid methyl esters were prepared according to the instructions for the Microbial Identification System (Microbial ID) and analysed using gas chromatography/mass spectrometry. Lipids were extracted and analysed according to Suresh *et al.* (2004). Respiratory quinones were extracted and analysed as described by Komagata & Suzuki (1987). For pigment characterization, cells were grown on 10×PGY-BG11 plates, scraped off the surface, suspended in 50 mM phosphate buffer or methanol and sonicated for 10 min at 4 °C. The suspension was centrifuged at 10 000 r.p.m. to remove unbroken cells. Absorption spectra were recorded for the supernatant with a Shimadzu UV-1601 spectrophotometer from 200 to 1100 nm. For *in vivo* pigment characterization, cells were grown aerobically under dark and light conditions at room temperature, suspended in 50% glycerol containing 0.04% sodium thioglycollate (to prevent oxidation of pigments) and the spectrum was recorded using 50% glycerol containing 0.04% sodium thioglycollate as a blank (Bryantseva *et al.*, 2000). The pigments were further resolved on HPLC with online diode array detection using a u-Bondapack C18 reverse-phase column. The solvent system employed was a gradient of 1 M ammonium acetate/methanol (1:4) (solvent A) to methanol (solvent B). The gradient used was 0% solvent A at time 0 to 5 min and, at 40 min, 100% solvent B. The flow rate used was 1 ml min⁻¹ and the detector was set at 519 nm.

DNA was isolated and the G+C content (mol%) was determined according to Reddy *et al.* (2000). A mean value from two independent experiments was calculated; the variation between the experiments was less than 2%. PCR amplification of approximately 1.5 kb of the *puf* gene was achieved and it was sequenced using the primer set PULM₁ (5'-KTTCGACTTCTGGGTSGG-3') and PULM₂ (5'-CCCATSGTCCAGCGCCAG-3') (Alarico *et al.*, 2002) and a strain of *Rhodospseudomonas* isolated in our lab was used as a control.

For 16S rRNA gene sequencing, DNA was prepared using the MoBio microbial DNA isolation kit (MoBio Laboratories). Approximately 1.5 kb of the 16S rRNA gene was amplified using primers GM3F (5'-AGAGTTTGATCMTGGC-3'; Nübel *et al.*, 1997) and 16S2 (5'-ACGGCTACCTTGTT-ACGACTT-3'; Reddy *et al.*, 2000). Fragments of around 1500 bp were purified from agarose gels with a Qiagen kit and sequenced using the primers 907R (5'-CCGTCAA-TTCCTTTTRAGTTT-3'; Nübel *et al.*, 1997), pC* (5'-CCCAGCTGCTGCCTCCCGTAG-3'), pE (5'-AAACTCAA-AGGAATTGACGG-3') and 16S2 (Reddy *et al.*, 2000). The phylogenetic position of CP2C^T was ascertained by aligning the partial sequence of the 16S rRNA gene containing 1453 nucleotides [nucleotides 6–1513 according to the *Escherichia coli* 16S rRNA (J 01695) numbering], with closely related sequences belonging to the *Alphaproteobacteria*, using the CLUSTAL W program (Thompson *et al.*, 1994). These sequences were included after the initial BLAST search. Pairwise evolutionary distances were computed

using the DNADIST program with the Kimura two-parameter model, as developed by Kimura (1980). Phylogenetic trees were constructed using three tree-making algorithms, neighbour-joining, unweighted pair group method with arithmetic averages (UPGMA) and DNA parsimony program (DNAPARS), from the MEGA2 package (Kumar *et al.*, 2001). Clade stability in the phylogenetic tree was assessed by analysing 1000 replicates of the dataset.

Strain CP2C^T forms colonies that are 1–2 mm in diameter, convex, rough and red-pigmented with rounded margins.

Fig. 1. Phase-contrast (a) and SEM (b) micrographs of cells of *Belnapia moabensis* CP2C^T. Bars, (a) 3 µm; (b) 1 µm.

Cells are coccoid, occur singly, in pairs and in groups (Fig. 1a, b) and are non-motile. They are catalase-, oxidase- and urease-positive, have C_{18:1}ω7c, C_{16:0}, C_{18:1} 2-OH and C_{16:1}ω7c/C_{15:0} iso 2-OH as the major fatty acids and have a DNA G + C content of 75 mol%. Other characteristics of CP2C^T are given in the species description. CP2C^T did not grow phototrophically under aerobic or anaerobic conditions on PNP or BS-V media (Saitoh & Nishimura, 1996). Methanolic (Fig. 2) or buffer extracts of the pigments or pigments recorded *in vivo* did not show any peaks characteristic of bacteriochlorophylls. In addition, PCR amplification of *puf* genes, using specific primers (Alarico *et al.*, 2002), resulted in no product, indicating that the biosynthetic genes for bacteriochlorophyll *a* (Bchl *a*) are absent in CP2C^T.

The pigment extracts were resolved into eight different peaks on HPLC; all of them exhibited absorption spectra characteristic of carotenoids. The major carotenoid had absorption maxima at 460, 490 and 520 nm.

The 16S rRNA gene sequence of CP2C^T contains all of the signature nucleotides that are characteristic of the *Alphaproteobacteria* and the strain could be grouped in the α-1 subgroup (Woese, 1987). A BLAST sequence similarity search, based on the 16S rRNA gene sequence, identified *Paracraurococcus ruber* (Saitoh *et al.*, 1998), *Muricoccus roseus* (Kämpfer *et al.*, 2003), *Roseomonas gilardii* (Rihs *et al.*, 1993), *Roseomonas mucosa* (Han *et al.*, 2003), *Rubritepida flocculans* (Alarico *et al.*, 2002) and *Roseococcus thiosulfatophilus* (Yurkov *et al.*, 1994) as related organisms. The evolutionary distances, calculated using the Kimura-2 factorial model and based on 16S rRNA gene sequences, indicated that CP2C^T is related to *Pcr. ruber* (94.9%), *M. roseus* (94.9%), *R. gilardii* (94.2%), *R. mucosa* (93.8%), *Rsc. thiosulfatophilus* (92.3%), *Teichococcus ludipueritiae* (92.3%), *Crc. roseus* (92.2%), *Rut. flocculans* (92.2%), *Rpi. globiformis* (91.5%) and *Acidiphilium cryptum* (90.7%) (see Supplementary Table S1 in IJSEM Online). As strain CP2C^T exhibited a 16S rRNA gene sequence similarity of less than 97.5% with all of these closely related species, CP2C^T does not represent a novel strain of any of these species

Fig. 2. Absorption spectrum of methanol extract of *Belnapia moabensis* CP2C^T.

(Stackebrandt & Goebel, 1994), but could represent a novel species belonging to one of the genera. A base-to-base comparison of 1447 nucleotides in the 16S rRNA gene sequences of strain CP2C^T and its relatives indicated a broad range of nucleotide variation: *Pcr. ruber* (80), *M. roseus* (83), *R. gilardii* (87), *R. mucosa* (94), *Rsc. thiosulfatophilus* (112), *T. ludipueritiae* (112), *Crc. roseus* (123), *Rut. flocculans* (120), *Rpi. globiformis* (137) and *A. cryptum* (144). In addition, CP2C^T exhibited differences of 47 nucleotides (*Pcr. ruber*) to 55 nucleotides (*R. mucosa* and *T. ludipueritiae*) in nucleotides that are highly conserved among these genera. Further, there were 14 nucleotides, T (between 218 and 221), G (382), T (593), G (594), C (647), A (648), A (672), T (1135), G (between 1137 and 1138), A (1141), C (1144), G (1261), A (1267) and T (1276), and three deletions at positions 99, 1010 and after 1463, that are present only in CP2C^T (numbering is with respect to the *E. coli* 16S rRNA gene sequence, J01695) (see Supplementary Table S2 in IJSEM Online). Thus, the phylogenetic signatures suggest that CP2C^T can be distinguished at a taxonomic rank higher than species, and probably at the genus level, from the other related species. In fact, some of the above genera have been distinguished mostly on differences at the phylogenetic level. For instance, the genus *Paracraurococcus* (Saitoh *et al.*, 1998) is phenotypically different from *Craurococcus* only with respect to maximum growth temperature and hydrolysis of Tween 80 (Saitoh *et al.*, 1998), but it showed sufficient differences in 16S rRNA gene sequence, having a similarity of only 94.7 %, to warrant separation. Similarly, *Muricoccus* (Kämpfer *et al.*, 2003) was delineated from *Roseomonas* (Rihs *et al.*, 1993; Han *et al.*, 2003) at a 16S rRNA gene sequence similarity of 95.9 %, though it exhibited differences only with respect to cell shape and fatty acids.

The topology of neighbour-joining and maximum-parsimony trees (Fig. 3 and Supplementary Fig. S1b in IJSEM Online) placed CP2C^T roughly equidistant from strains containing Bchl *a*, (*Pcr. ruber*, *Crc. roseus*, *Rut. flocculans* and *Rsc. thiosulfatophilus*) and lacking Bchl *a* (*M. roseus*, *R. gilardii* and *R. mucosa*). In UPGMA trees (Supplementary Fig. S1a in IJSEM Online), CP2C^T clustered with the Bchl *a*-containing *Pcr. ruber*. The above results clearly indicate that the position of CP2C^T is sufficiently distinct and deeply rooted from the strains containing or lacking Bchl *a* to warrant the creation of a novel genus.

Apart from phylogenetic characteristics, the phenotypic properties of CP2C^T also suggest that the strain is quite different from its close relatives. For example, CP2C^T is different from *Pcr. ruber* at the genus level in that it does not contain Bchl *a*, does not produce H₂S and contains significant levels of fatty acid C_{17:1}ω7c (Table 1). Additional differences are listed in Table 1. Strain CP2C^T is also closely related to *M. roseus* on the basis of rRNA gene sequence similarity (94.9 %; see Supplementary Table S1 in IJSEM Online). Both organisms lack Bchl *a*, but differ in cell morphology (Table 1), a feature sufficient to differentiate them at the genus level (Yoon *et al.*, 2001), and in the lack of

Fig. 3. Neighbour-joining tree based on 16S rRNA gene sequences (1453 bp) showing the phylogenetic relationship between *Belnapia moabensis* CP2C^T and other related members of the Alphaproteobacteria. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are indicated at the nodes. Bar, 0.02 substitutions per nucleotide position.

lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase activities (Table 1) and C_{12:0} fatty acids. The neighbour-joining tree indicated that CP2C^T was most closely related to *Rsc. thiosulfatophilus*, a result further supported by in-depth 16S rRNA gene sequence comparisons of signature nucleotides (see Supplementary Table S2 in IJSEM Online). However, CP2C^T is different from *Rsc. thiosulfatophilus* in that it is non-motile, does not contain Bchl *a* and is a mesophile (Table 1). CP2C^T can be differentiated from the closely related species *R. gilardii* and *R. mucosa* on the basis of fatty acid content. CP2C^T had the highest DNA G + C content, 75 mol%, of all the bacteria used in the present comparison (which ranged from 61 to 71 mol%). Additional differentiating traits are listed in Table 1.

In conclusion, CP2C^T can be differentiated from the closest nine genera on the basis of phylogenetic signature sequences, the absence of Bchl *a*, fatty acid content, DNA G + C content and physiology (Table 1). Since CP2C^T does not possess the generic characteristics of any of the nine closest genera, we suggest a novel genus to accommodate CP2C^T, for which the name *Belnapia* gen. nov. is proposed. Strain CP2C^T represents the type strain of the type species of this genus, *Belnapia moabensis* sp. nov. Strain CP2C^T has several interesting physiological characteristics as it is oligotrophic, salt-sensitive (does not grow in presence of 0.5 % NaCl), grows on 0.5 % phenanthrene and can use oxalate as sole carbon source.

Description of *Belnapia* gen. nov.

Belnapia (Bel.na'pi.a. N.L. fem. n. *Belnapia* after J. Belnap, in honour of her contributions to the study of BSCs).

Table 1. Characteristics that differentiate *Belnapia moabensis* CP2C^T from closely related organisms

Taxa: 1, *Belnapia moabensis* CP2C^T; 2, *Pcr. ruber* NS89^T (data from Saitoh *et al.*, 1998; Saitoh & Nishimura, 1996); 3, *Crc. roseus* NS130^T (Saitoh *et al.*, 1998; Saitoh & Nishimura, 1996); 4, *Rsc. thiosulfatophilus* RB3^T (Yurkov *et al.*, 1994); 5, *R. gilardii* ATCC 49956 (Rihs *et al.*, 1993; Han *et al.*, 2003); 6, *R. mucosa* MDA 5527^T (Han *et al.*, 2003); 7, *M. roseus* DSM 14916^T (Kämpfer *et al.*, 2003); 8, *T. ludipueritiae* DSM 14915^T (Kämpfer *et al.*, 2003); 9, *Rut. flocculans* DSM 14296^T (Alarico *et al.*, 2002). –, Negative; +, positive; v, variable; w+, weakly positive; ND, not determined; S, susceptible; R, resistant; tr, trace; NK, not known.

Characteristic	1	2	3	4	5	6	7	8	9
Morphology									
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Short rods	Short rods	Short rods
Colony colour	Red	Red	Pink	Pink	Pink	Pink	Pink	Pink	Red
Motility	–	–	–	+	v	+	–	–	+
Growth range (°C)	15–30	20–42	20–37	NK	30–42	25–42	20–42	20–42	30–50
Optimum temperature (°C)	25	30	30	30	35	35	30	30	50
Biochemical									
Arginine dihydrolase	–	ND	ND	ND	–	ND	+	+	ND
Bchl <i>a</i>	–	+	+	+	–	–	–	–	+
DNA G+C content (mol%)	75	71	70.5	70.4	67.6	66.3	68.9	65.8	70.2
H ₂ S production	–	+	+	ND	–	ND	–	–	ND
Lipase	–	–	+	–	ND	ND	ND	ND	–
Lysine decarboxylase	–	–	–	ND	–	ND	+	+	ND
Nitrate reduction	+	+	+	ND	–	+	ND	ND	–
Ornithine decarboxylase	–	–	–	ND	–	ND	+	+	ND
Starch hydrolysis	–	+	+	–	ND	ND	ND	ND	–
Acid production from:									
Arabinose	–	+	+	ND	ND	ND	–	–	ND
Fructose	–	–	+	ND	ND	ND	ND	ND	ND
Galactose	–	–	+	ND	ND	ND	ND	ND	ND
Glucose	–	+	+	ND	ND	ND	–	–	ND
Xylose	–	+	+	ND	–	ND	–	–	ND
Utilization of carbon sources:									
Acetate	–	–	–	+	ND	ND	+	+	–
Adonitol	+	ND	ND	ND	ND	ND	–	–	ND
Alanine	–	+	+	w+	ND	ND	–	–	w+
Arabinose	–	+	+	–	ND	+	–	–	+
Cellobiose	+	ND	ND	ND	ND	ND	–	–	ND
Citrate	–	–	–	+	ND	+	–	–	–
Cysteine	–	+	+	–	ND	ND	ND	ND	+
Fructose	–	+	+	–	ND	ND	w+	+	–
Fumarate	+	+	+	–	ND	ND	–	ND	+
Galactose	–	+	+	–	ND	ND	–	–	–
Glucose	+	+	+	w+	ND	+	–	w+	+
Glutamic acid	–	+	+	ND	ND	ND	ND	ND	+
Glycerol	–	+	+	ND	ND	ND	–	–	+
Inositol	+	ND	ND	ND	ND	ND	–	–	ND
Maltose	+	ND	ND	ND	ND	–	–	–	ND
Mannitol	+	–	–	ND	ND	–	–	–	ND
Mannose	+	+	+	–	ND	ND	–	–	–
Methionine	+	–	–	ND	ND	ND	ND	ND	ND
Proline	+	ND	ND	ND	ND	ND	–	–	ND
Pyruvate	–	+	+	+	ND	ND	–	–	+
Rhamnose	+	+	+	–	ND	ND	–	–	ND
Ribose	+	+	+	w+	ND	ND	–	–	–
Sorbitol	+	–	–	ND	ND	ND	–	–	ND
Sorbose	+	+	ND	–	ND	ND	–	–	+
Sucrose	+	ND	ND	ND	ND	ND	–	–	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9
Trehalose	+	ND	ND	ND	ND	ND	W+	—	ND
Xylose	+	+	+	W+	ND	ND	—	—	+
Antibiotic sensitivity:									
Gentamicin	S	ND	ND	R	S	ND	ND	ND	ND
Penicillin	S	ND	ND	R	ND	ND	ND	ND	ND
Polymyxin B	R	ND	ND	S	ND	ND	ND	ND	ND
Tetracycline	R	ND	ND	S	S	ND	ND	ND	ND
Vancomycin	R	ND	ND	S	ND	ND	ND	ND	ND
Fatty acid methyl esters*									
Saturated:									
C _{12:0}	—	1.0	—	2.3	—	—	5.0	—	2.5
C _{14:0}	—	0.6	1.5	—	—	—	1.0	0.9	0.3
C _{16:0}	17.02	8.7	12.5	7.6	16.93	19.53	16.6	6.9	20.0
C _{18:0}	2.04	1.3	2	0.7	1.15	1.16	1.0	—	26.6
Branched:									
C _{15:0} iso	0.84	—	—	—	—	—	—	—	—
Unsaturated:									
C _{16:1} ω 7c	7.67	5.8	12.7	0.9	1.3	1.23	22.0	3.3	1.2
C _{16:1} ω 5c	1.28	0.4	1.1	0.5	0.23	—	—	tr	—
C _{17:1} ω 7c	3.20	—	—	—	—	—	2.1	—	—
C _{18:1} ω 7c	53.78	73.4	53.2	65.1	60.64	46.55	39.2	68.3	23.4
C _{18:1} ω 5c	1.71	—	0.6	1.1	0.27	—	0.9	tr	0.5
C _{18:2} ω 6,9c/C _{18:0} ante	—	—	—	—	0.73	0.4	—	—	—
Hydroxy:									
C _{14:0} 3-OH	2.85	0.8	1.1	3.0	0.65	0.89	—	tr	2.3
C _{16:0} 2-OH	—	—	—	—	—	1.24	—	—	2.7
C _{16:0} 3-OH	1.41	—	—	—	2.86	4.13	—	—	—
C _{18:1} 2-OH	7.61	6.2	12.3	15.5	11.26	3.82	8.8	15.1	0.7
C _{18:0} 3-OH	0.58	0.6	1.8	2.4	—	—	1.0	—	3.4
C _{19:0} 2-OH 11,12	—	—	—	—	—	—	—	—	21.6
Unknown:									
Unknown 10.928	2.85	—	—	—	—	—	—	—	—
C _{19:0} 10-methyl	—	—	—	—	—	—	1.1	—	—
C _{19:0} cyclo ω 8c	—	—	—	—	4.19	21.6	—	4.3	10.6

*FAME data from Kämpfer *et al.* (2003), Han *et al.* (2003) and Alarico *et al.* (2002).

Gram-negative cocci, non-motile, red-pigmented by carotenoids, do not contain Bchl *a*, aerobic, facultatively fermentative. Positive for catalase, oxidase and urease but test negative for lysine and ornithine decarboxylases and arginine dihydrolase. Do not hydrolyse cellulose or starch. Contain C_{18:1} ω 7c and C_{16:0} as the major fatty acids, phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol (cardiolipin) as polar lipids and ubiquinone-9 as the respiratory quinone. DNA G + C content is 75 mol%. Phylogenetically, a member of the α -1 subgroup of the *Alphaproteobacteria*. The type species is *Belnapia moabensis*.

Description of *Belnapia moabensis* sp. nov.

Belnapia moabensis (mo.a.ben'sis. N.L. gen. n. *moabensis* of Moab, UT, USA, where the type strain was isolated).

Shows the following properties in addition to those given for the genus. Colonies are convex, round and rough. It grows from 15 to 30 °C, but not at 37 °C, with an optimum growth temperature of 25 °C. The pH range for growth is 6–8, with an optimum pH of 7. Cannot tolerate NaCl. Cells test negative for lipase, phosphatase, β -galactosidase, gelatinase, DNase, arginine decarboxylase and phenylalanine deaminase activities. Negative in methyl red, Voges–Proskauer, indole and Simmons' citrate tests. Does not hydrolyse casein, cellulose, aesculin or starch and does not produce H₂S gas, but reduces nitrate to nitrite. Cells do not produce acid from D-fructose, L-arabinose, D-galactose, D-glucose, glycerol, D-maltose, D-mannitol, sucrose, D-sorbitol or D-xylose. Ferments L-arabinose, D-galactose, D-maltose and D-xylose but not D-fructose, D-glucose, lactose, sucrose, D-mannose or D-sorbitol. Utilizes adonitol, D-cellobiose,

glucose, dulcitol, fumaric acid, D-glucose, *meso*-inositol, inulin, lactose, lactic acid, maltose, D-levulose, D-mannitol, D-mannose, D-raffinose, D-ribose, L-rhamnose, L-sorbose, sucrose, D-sorbitol, D-trehalose, D-xylose, L-asparagine, L-isoleucine, L-methionine, L-proline, L-threonine, L-valine, oxalate and phenanthrene as sole carbon sources but not L-arabinose, acetate, citrate, dextran, ethanolamine, D-fructose, fumaric acid, D-galactose, glycerol, D-melibiose, pyruvate, succinate, L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glycine, L-glutamine, L-glutamic acid, L-histidine, L-leucine, L-lysine, L-phenylalanine, L-serine, L-tryptophan, L-tyrosine, adenine, cytosine, guanine, thymidine, nicotinic acid, tartaric acid or indole. Cells are sensitive to carbencillin (100 µg disc), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), erythromycin (2 µg), gentamicin (10 µg), novobiocin (30 µg), penicillin (10 U), rifampicin (30 µg), streptomycin (10 µg), sulfisoxazole (300 µg) and sulfathiazole (300 µg), but resistant to aztreonam (30 µg), bacitracin (10 U), cephalothin (30 µg), colistin (10 µg), ethambutol (50 µg), ethionamide (25 µg), nitrofurantoin (150 µg), polymyxin B (300 U), tetracycline (30 µg), trimethoprim (5 µg) and vancomycin (30 µg). Methanol extracts of the type strain exhibit absorption maxima at 315, 363, 465, 493 and 519 nm. Phosphate buffer suspensions (0.1 M, pH 7.4) absorb maximally at 354, 415, 465, 505 and 536 nm.

The type strain, strain CP2C^T (= ATCC BAA-1043^T = DSM 16746^T), was isolated from a BSC in the Colorado Plateau, USA.

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