Dyadobacter crusticola sp. nov., from biological soil crusts in the Colorado Plateau, USA, and an emended description of the genus *Dyadobacter* Chelius and Triplett 2000

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Bacterial strain CP183-8^T was isolated from biological soil crusts collected in the Colorado Plateau, USA. Cells of this strain were aerobic, non-motile, Gram-negative, psychrotolerant and formed beaded chains in the stationary growth phase. They contained C_{16:1}ω5c and C_{16:1}ω7c as major fatty acids. 16S rRNA gene sequence analysis assigned the strain to the genus *Dyadobacter*. However, it shared a sequence similarity of only 95.88% with the type strain of *Dyadobacter fermentans*, NS114^T. Because it also exhibited a significant number of phenotypic and chemotaxonomic differences from *D. fermentans*, it is described as a novel second species in the genus *Dyadobacter*, with the name *Dyadobacter crusticola* sp. nov. The type strain is CP183-8^T (=DSM 16708^T = ATCC BAA-1036^T).

Biological soil crusts (BSCs) are the topmost layers of the soil (millimetres to centimetres in thickness) that are composed of mineral substrates held together by complex assemblages of micro-organisms that include cyanobacteria, eukaryotic green algae, fungi, lichens, mosses and heterotrophic bacteria. BSCs stabilize the soil against erosion (Belnap & Gardner, 1993) and import nutrients such as carbon and nitrogen (Belnap, 2002; Johnson *et al.*, 2005). Biological crusts may cover a large portion of arid and semi-arid landscapes in the south-west USA (Belnap, 1994), where the higher plant cover is restricted. In spite of their ecological relevance, bacterial members of the community other than cyanobacteria are very poorly known. The arid lands of the Colorado Plateau are unique because of the plateau's high altitude and are characterized by low humidity, greater penetration of solar (UV and visible) radiation, freezing and generally very low winter temperatures, low rainfall (175–300 mm) and high air and ground temperatures in summer (Bowker *et al.*, 2002). As a result of these extreme conditions, the microbial communities on the top layers of the soils encounter a variety of stresses that may have led to unique survival adaptations. It is well established

that the upper layers of BSCs from the Colorado Plateau are dominated by cyanobacteria (Bowker *et al.*, 2002; Garcia-Pichel *et al.*, 2001; Yeager *et al.*, 2004) that colonize the topsoils and help in the formation of these crusts. The predominant cyanobacteria associated with BSCs from the Colorado Plateau are *Microcoleus vaginatus* and *Microcoleus steenstrupii* (Garcia-Pichel *et al.*, 2001; Yeager *et al.*, 2004). Recently, the existence of a wide variety of heterotrophic bacteria belonging to the *Actinobacteria*, *Proteobacteria*, *Bacterioidetes*, Gram-positive bacilli, *Acidobacteria* and *Thermomicrobia* was demonstrated by both culture-dependent and culture-independent methods (G. S. N. Reddy and F. Garcia-Pichel, unpublished). However, most of these bacteria have not been characterized. In the present study one particular isolate, designated CP183-8^T, assigned to the genus *Dyadobacter* was subjected to polyphasic characterization.

The genus *Dyadobacter* was described by Chelius & Triplett (2000) to accommodate Gram-negative, rod-shaped cells that are straight to curved, occurring in pairs in young cultures and forming chains of coccoid cells in old cultures. The type strain of the only species, *Dyadobacter fermentans* NS114^T, was isolated from surface-sterilized *Zea mays* stems. Cells of *D. fermentans* are non-motile, oxidase- and catalase-positive, producing a non-diffusible yellow, flexirubin-like pigment; they are aerobic and chemo-organotrophic, capable of fermenting glucose and sucrose, but not being able to hydrolyse cellulose or starch. The G + C content of the DNA is 48 mol%.

Strain CP183-8^T was isolated from BSC samples collected from the Colorado Plateau (38° 09' 839" N 109° 44' 560" W)

Published online ahead of print on 7 January 2005 as DOI 10.1099/ijls.0.63498-0.

Abbreviation: BSC, biological soil crust.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of CP183-8^T is AJ821885.

A comparison of nucleotides of the 16S rRNA gene sequence that differentiate CP183-8^T and *Dyadobacter fermentans* NS114^T is available as a supplementary table in IJSEM Online.

in May 2003 (G. S. N. Reddy and F. Garcia-Pichel, unpublished). The medium used for isolation was BG11-PGY (10 % BG-11 mineral medium, 0.25 % peptone, 0.25 % yeast extract, 0.25 % glucose, 1.5 % agar). The composition of BG11 base was: 1.5 g NaNO₃, 40 mg K₂HPO₄·3H₂O, 75 mg MgSO₄·7H₂O, 36 mg CaCl₂·2H₂O, 6 mg citric acid, 6 mg ferric ammonium citrate, 1 mg EDTA (disodium magnesium), 20 mg Na₂CO₃, 1 ml trace metal solution in 1 l Milli-Q water, pH 7.4 (the composition of the trace metal solution is as given in Rippka *et al.*, 1979). For maintenance, 10 × PGY medium on BG11 base was used. Morphological characteristics were determined using the phase-contrast microscope and scanning electron microscope. For determination of biochemical characteristics, cultures were grown at 25 °C in 10 × BG11-PGY medium and tests were performed as described by Lanyi (1987) and Smibert & Krieg (1994). The ability of the culture to utilize a carbon compound as the sole carbon source was checked by adding each carbon compound at a final concentration of 0.5 % to a base of BG11 medium without citric acid. The sensitivity of the culture to different antibiotics was checked using antibiotic discs supplied by Becton Dickinson Microbiological Systems.

Cells were grown on trypticase soy agar at 25 °C and the fatty acid methyl esters were characterized as described by Reddy *et al.* (2002) and Sato & Murata (1988). The presence of flexirubin-like pigments was tested spectrophotometrically as described by Güde (1980). Initially, a drop of 20 % KOH was added to a single colony of strain CP183-8^T and the change in colony colour from yellow to orange and then to red was observed (Güde, 1980). Cells of CP183-8^T were grown on 10 × BG11-PGY agar medium, scraped off and suspended in absolute ethanol and extracted by vortexing. After removing cell debris by centrifugation at 6000 r.p.m. for 5 min, a UV-visible spectrum was recorded from 250 to 700 nm in alcohol. To the same extract, 20 % KOH was added to a final concentration of 1 % and a new spectrum was then recorded. Polar lipids were extracted and analysed according to the method described by Komagata & Suzuki (1987). The 16S rRNA gene from CP183-8^T was amplified using primers GM3F (5'-AGAGTTTGATCMTGGC-3') and 16S2 (5'-ACGGCTACCTTGTACGACTT-3') (Nübel *et al.*, 1997; Reddy *et al.*, 2000). Fragments of about 1500 bp were purified from agarose gels by using a Qiagen kit and then sequenced using the primers 907R (5'-CCGTCAATTCCTTTTRAGTTT-3') (Nübel *et al.*, 1997), pC* (5'-CCC-ACTGCTGCCTCCCGTAG-3'), pE (5'-AAACTCAAAGG-AATTGACGG-3') and 16S2 (Reddy *et al.*, 2000). The 16S rRNA gene sequence of CP183-8^T was aligned with closely related sequences retrieved from the EMBL database using CLUSTAL W (Thompson *et al.*, 1994). Pairwise evolutionary distances were computed using the Kimura two-parameter method (Kimura, 1980). Phylogenetic trees were constructed using the tree-making algorithms UPGMA (unweighted pair group method with arithmetic averages), neighbour-joining and DNA parsimony of the MEGA 2 package (Kumar *et al.*,

2001) and the stability among the clades in the phylogenetic tree was assessed by using 1000 replicates.

Morphological, growth, biochemical and chemotaxonomic characteristics of strain CP183-8^T are given in the species description below. Cells of CP183-8^T were Gram-negative, formed chains of coccoid cells in stationary phase (Fig. 1) and contained C_{16:1}ω5c and C_{16:1}ω7c as major fatty acids. Based on these characteristics, CP183-8^T was assigned to the genus *Dyadobacter* (Chelius & Triplett, 2000). Cells of CP183-8^T changed from yellow to orange upon addition of 20 % KOH solution, indicating that they contain a flexirubin-type pigment (Weeks, 1981). Further evidence for this was derived from the UV-visible spectrum; the strain exhibited three peaks characteristic of flexirubin at 428, 452 and 478 nm in ethanol (Fig. 2) (Chelius & Triplett, 2000). A peak at 329.5 nm upon deprotonation using 1 % KOH as well as the broadening of peaks with time were also observed. The presence of a flexirubin-type pigment in cells of strain CP183-8^T supports its inclusion within the genus *Dyadobacter*. A sequence similarity search by BLAST analysis using the almost complete 16S rRNA gene sequence (1434 nucleotides, base positions 24–1462 with respect to the *Escherichia coli* numbering system) of CP183-8^T also identified *D. fermentans* as its closest relative. The topology of the phylogenetic tree (Fig. 3) confirmed the evolutionary relatedness of CP183-8^T to the type strain of *D. fermentans* (NS114^T); it formed a robust cluster with a bootstrap resampling value of 100 %. However, the evolutionary distance, as calculated by using the Kimura two-parameter model, indicated that strain CP183-8^T shared a maximum 16S rRNA gene sequence similarity of 95.88 % with *D. fermentans*, suggesting that it represents a separate species. DNA–DNA relatedness studies between

Fig. 1. Scanning electron micrograph of cells of *Dyadobacter crusticola* sp. nov. CP183-8^T. Bar, 0.2 µm.

Fig. 2. Absorption spectra of ethanol (solid line) and alkaline ethanol (dotted line) extracts of *Dyadobacter crusticola* sp. nov. CP183-8^T.

CP183-8^T and *D. fermentans* were not carried out as a strain that exhibits a difference of more than 2.5 % at the 16S rRNA level is unlikely to have a relatedness of more than 70 % at the whole genome level (Stackebrandt & Goebel, 1994). Furthermore, nucleotide base-to-base comparison of

16S rRNA gene sequences of CP183-8^T and the type strain of *D. fermentans* revealed that the genus *Dyadobacter* contains three variable regions: region I, 182–207 (25 bases); region II, 590–649 (59 bases); and region III, 835–848 (13 bases) (details are given in a supplementary table in IJSEM Online). The sequence of CP183-8^T exhibited a difference of 60/1434 nt in total and 14/25, 12/59 and 10/13 nt, respectively, in regions I, II and III. This indicates that CP183-8^T is sufficiently different from *D. fermentans* to merit separate species status within the genus *Dyadobacter*.

Strain CP183-8^T also exhibited several differences at the phenotypic level. In contrast to *D. fermentans*, CP183-8^T was psychrotolerant (it could grow from 5 to 30 °C but not at 37 °C), could not produce acid from glucose, ribose or sucrose, did not oxidize or ferment glucose or sucrose and could not utilize acetate, arabinose, galactose, glycerol, mannitol, mannose, rhamnose, sorbose or tartaric acid as sole carbon source (Table 1). Strain CP183-8^T contained C_{14:0}, iso-C_{15:1}, C_{18:0} and C_{18:1} as additional fatty acids; it also contained increased amounts of C_{16:0} and reduced amounts of C_{16:0} 3-OH and iso-C_{17:0} 3-OH fatty acids (Table 2) compared with *D. fermentans* NS114^T.

In contrast to the generic characteristics of the genus *Dyadobacter* as described by Chelius & Triplett (2000), strain CP183-8^T could not ferment glucose or sucrose.

Fig. 3. Neighbour-joining tree based on 16S rRNA (1434 bases) gene sequence analysis, showing the phylogenetic relationships between *Dyadobacter crusticola* sp. nov. CP183-8^T, *D. fermentans* NS114^T and other related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. Bar, 0.05 substitutions per nucleotide position.

Table 1. Phenotypic characteristics that differentiate *Dyadobacter crusticola* sp. nov. from *D. fermentans*

Strains: 1, *D. crusticola* CP183-8^T; 2, *D. fermentans* NS114^T (data from Chelius & Triplett, 2000).

Characteristic	1	2
Growth temperature		
5 °C	+	–
10 °C	+	–
37 °C	–	+
Acid-gas production		
Glucose	–	+
Ribose	–	+
Sucrose	–	+
Utilization of carbon compounds		
Acetate	–	+
Arabinose	–	+
Galactose	–	+
Glycerol	–	+
Mannitol	–	+
Mannose	–	+
Rhamnose	–	+
Sorbose	–	+
Tartaric acid	–	+
Oxidation and fermentation		
Glucose	–	+
Sucrose	–	+

Table 2. Comparison of the fatty acid compositions of *Dyadobacter crusticola* sp. nov. and *D. fermentans*

Strains: 1, *D. crusticola* CP183-8^T; 2, *D. fermentans* NS114^T (data from Chelius & Triplett, 2000). Values are percentages of total fatty acid content.

Fatty acid	1	2
C _{14:0}	1.4	—
iso-C _{15:1}	1.5	—
iso-C _{15:0}	13.4	16.8
C _{16:1} ω5c	21.4	17.5
C _{16:1} ω7c	41.2	43.5
C _{16:0}	12.4	4.76
iso-C _{15:0} 3-OH	2.4	2.74
Unknown	0.3	1.32
C _{16:0} 3-OH	2.2	4.66
C _{18:0}	0.16	—
C _{18:1}	0.83	—
iso-C _{17:0} 3-OH	2.9	7.38

However, other phenotypic, chemotaxonomic and genotypic characteristics support the placement of CP183-8^T in the genus *Dyadobacter* as *Dyadobacter crusticola* sp. nov. Therefore, the description of the genus *Dyadobacter* needs to be emended with respect to the fermentation of sugars. The genus *Dyadobacter* belongs to the 'Flexibacter group' within the phylum *Bacterioidetes*. It is interesting to note that the members of the phylum *Bacterioidetes* are very common inhabitants of the BSCs from the Colorado Plateau (G. S. N. Reddy and F. Garcia-Pichel, unpublished) as well as the Sonoran desert (M. Nagy, A. Perez and F. Garcia-Pichel, unpublished), temperate coastal dune crusts in the Cape Cod National Seashore (Smith *et al.*, 2004) and gypsum crusts from Antarctica (Hughes & Lawley, 2003). Thus, *D. crusticola* may be an important component of the BSCs, and may have an important role in nutrient cycling (as it produces extracellular lipase and phosphatase) and gluing together of soil particles (as it secretes exopolysaccharides).

Emended description of the genus *Dyadobacter* Chelius and Triplett 2000

Gram-negative rods in straight to curved arrangements, occurring in pairs in young cultures and forming chains of coccoid cells in old cultures. Cells are non-motile, aerobic, oxidase and catalase-positive, produce a non-diffusible, yellow, flexirubin-like pigment and contain C_{16:1}ω5c and C_{16:1}ω7c as the major fatty acids. They do not hydrolyse cellulose or starch and the G+C content of the DNA is 48 mol%.

Description of *Dyadobacter crusticola* sp. nov.

Dyadobacter crusticola (crus.ti'co.la. L. n. *crusta* crust; L. suff. *-cola* dweller; N.L. n. *crusticola* a dweller of crust).

Colonies are yellow, mucoid, convex, round and smooth.

Cells stain Gram-negative, are non-motile, curved to straight rods, straight to V-shaped and few were beaded rods. Grows at 5–30 °C (but not at 37 °C) and is thus psychrotolerant in nature, with an optimum growth temperature of 25 °C. The pH range for growth is 6–8 (optimum 7), and it can tolerate up to 1 % NaCl. Cells are positive for catalase, oxidase, lipase, phosphatase and β-galactosidase, and negative for urease, gelatinase, DNase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase and arginine dihydrolase. Does not hydrolyse casein, cellulose or starch but can hydrolyse aesculin weakly. Also negative for methyl red, Voges–Proskauer reaction, indole and Simmons' citrate tests. Does not produce H₂S gas and does not reduce nitrate to nitrite. Cells produce acid from D-fructose but not from L-arabinose, D-galactose, D-glucose, lactose, D-maltose, D-mannitol, sucrose, D-sorbitol or D-xylose. Able to ferment L-arabinose, D-galactose, D-maltose and D-xylose but not D-fructose, D-glucose, lactose, sucrose, D-mannose or D-sorbitol. Is able to utilize D-cellobiose, glucose, dulcitol, D-glucose, *meso*-inositol, inulin, lactose, lactic acid, D-laevulose, D-mannitol, D-melibiose, D-raffinose, D-ribose, sucrose, D-sorbitol, D-trehalose and D-xylose as sole carbon sources but not adonitol, L-arabinose, acetate, citrate, dextran, ethanolamine, D-fructose, fumaric acid, D-galactose, glycerol, D-mannitol, D-mannose, pyruvate, L-rhamnose, L-sorbose, succinate, L-alanine, L-arginine, L-aspartic acid, L-asparagine, L-cysteine, L-glycine, L-glutamine, L-glutamic acid, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, adenine, cytosine, guanine, thymidine, nicotinic acid, oxalate, tartaric acid, indole or phenanthrene. Cells are sensitive to (per disc): carbenicillin (100 µg), doxycycline (30 µg), novobiocin (30 µg), polymyxin B (300 U), rifampicin (30 µg) and tetracycline (30 µg) but resistant to azithromycin (15 µg), aztreonam (30 µg), bacitracin (10 units), ceftriaxone (30 µg), chloramphenicol (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), colistin (10 µg), erythromycin (2 µg), ethambutol (50 µg), gentamicin (10 µg), nitrofurantoin (150 µg), penicillin (10 U), streptomycin (10 µg), sulfisoxazole (300 µg), sulfthiazole (300 µg), trimethoprim (5 µg) and vancomycin (30 µg). The pigment present is a flexirubin type with absorption maxima at 428, 452 and 478 nm. The fatty acids and their percentage contributions are listed in Table 2; polar lipids present are phosphatidyl serine, phosphatidylglycerol and diphosphatidylglycerol (cardiolipin).

The type strain is CP183-8^T (= DSM 16708^T = ATCC BAA-1036^T), isolated from a BSC sample collected from the Colorado Plateau.

Acknowledgements

This research was funded by the National Science Foundation Biotic Surveys and Inventories grant 0206711, to F. G.-P.

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