Tsukamurella carboxydivorans sp. nov., a carbon monoxide-oxidizing actinomycete

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A Gram-positive, slightly acid–alcohol-fast, carbon monoxide-oxidizing bacterium, strain Y2^T, was isolated from a soil sample collected from a roadside in Seoul, Korea. On the basis of 16S rRNA gene sequence comparative analyses, strain Y2^T was shown to belong to the genus *Tsukamurella* and was most closely related to *Tsukamurella tyrosinosolvens* DSM 44234^T (GenBank accession no. AY238514; 99.8%). The predominant fatty acids were C_{18:1}ω9c and C_{16:0}. The cell-wall peptidoglycan of strain Y2^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid. Strain Y2^T contained galactose and arabinose as the whole cell sugars. The DNA G + C content was 77 mol%. The DNA–DNA relatedness value between strain Y2^T and *T. tyrosinosolvens* DSM 44234^T was 62.7%. Based on the combination of the carbon source utilization pattern, fatty acid profile, cell-wall chemotype, DNA G + C content and DNA–DNA hybridization experiments, it is proposed that strain Y2^T (=KCCM 42885^T=JCM 15482^T) represents the type strain of a novel species, *Tsukamurella carboxydivorans* sp. nov.

The genus *Tsukamurella* was first proposed by Collins *et al.* (1988) to accommodate a group of mycolic acid-containing actinomycetes that exhibited very high similarities in their 16S rRNA sequences. Members of the genus contain a series of very long chain (62 to 78 carbons), highly unsaturated (one to six double bonds) mycolic acids (Collins *et al.*, 1988), in addition to possessing *meso*-diaminopimelic acid and arabinogalactan. At the time of writing, the genus comprises nine species with validly published names. One member of the genus, *Tsukamurella wratislaviensis* has been transferred to the genus *Rhodococcus* (Kattar *et al.*, 2001). Among the remaining species, four of them have been isolated from clinical specimens. Two species have been associated with foaming that caused several operational problems in activated sludge plants (Goodfellow *et al.*, 1998). One species, *Tsukamurella paurometabola* (Collins *et al.*, 1988), was isolated from the mycetomes and ovaries of the bed bug. *Tsukamurella spongiae* (Olson *et al.*, 2007) was isolated from a deep-water marine sponge. Species isolated from clinical specimens include *Tsukamurella incheonensis* (Yassin *et al.*, 1995), *Tsukamurella pulmonis* (Yassin *et al.*, 1996),

Tsukamurella strandjordii (Kattar *et al.*, 2001) and *Tsukamurella tyrosinosolvens* (Yassin *et al.*, 1997). Species of the genus associated with activated sludge comprise *Tsukamurella pseudospumae* (Nam *et al.*, 2004) and *Tsukamurella spumae* (Nam *et al.*, 2003).

Strain Y2^T, an aerobic bacterium able to grow at a low concentration of carbon monoxide (CO, 400 p.p.m.) at 30 °C, was isolated from subsurface (10 cm depth) soil samples collected from the side of a busy road at Yonsei University, Seoul, Korea, using two enrichment steps as described previously (Park *et al.*, 2008). Although strain Y2^T was isolated under a low CO concentration, the novel strain was also able to grow in mineral medium (Kim & Hegeman, 1981) at a high concentration of CO (300 000 p.p.m.). Crude cell-free extracts were prepared from cells grown at 30 °C under a gas mixture of 400 p.p.m. or 300 000 p.p.m. CO in air according to the method of Park *et al.* (2003). The enzyme CO-dehydrogenase (CO-DH) was assayed photo-metrically by measuring the CO-dependent reduction of 2-(4-indophenyl)-3-(4-nitrophenyl)-2H-tetrazolium chloride (INT, ϵ_{496} =17.981 mM⁻¹ cm⁻¹) (Kraut *et al.*, 1989). Crude extracts prepared from cells of strain Y2^T grown under either low or high CO concentrations exhibited CO-DH activity but the activity was higher in the extracts prepared from cells grown under low CO concentrations [8.7 nmol min⁻¹ (mg protein)⁻¹ INT reduced] than from the extracts prepared from high CO-grown cells [3.2 nmol min⁻¹ (mg protein)⁻¹ INT reduced].

Total genomic DNA from strain Y2^T was isolated by using cetyltrimethylammonium bromide as described by Ausubel

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Abbreviations: CO-DH, CO-dehydrogenase; INT, 2-(4-indophenyl)-3-(4-nitrophenyl)-2H-tetrazolium chloride.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Tsukamurella carboxydivorans* Y2^T is EU521689.

A supplementary table detailing the fatty acid contents of strain Y2^T and other closely related species of the genus *Tsukamurella* is available with the online version of this paper.

et al. (1995). The partial CO-DH large subunit gene was amplified with primers BMSf (5'-GGCGGCTTYGGST-CSAAGAT-3') and O/Br (5'-YTCGAYGATCATCGGRTT-GA-3') on a Master gradient PCR machine (Eppendorf) as described by King (2003). The amplified DNA fragments (approx. 1250 bp) were purified from agarose gels using a gel extraction kit (iNtRON Biotechnology) and sequenced. Sequencing of the PCR product revealed that strain Y2^T had a partial CO-DH large subunit gene (GenBank accession no. EU723833) that was most closely related to the CO-DH large subunit gene of *Mesorhizobium* sp. NMB1 (AY307905; 82.0 %).

Gram-staining and acid-alcohol fast staining were performed by following the procedures of Gerhardt *et al.* (1994) and Ebersole (1992), respectively. The ability of the organism to grow on various carbon sources was determined as described by Yassin *et al.* (1995). Tests to determine the decomposition of casein, aesculin and urea were performed according to the methods of Gordon & Mihm (1957), Gordon (1966) and Paliwal & Randhawa (1977), respectively. Hydrolysis of hypoxanthine, xanthine and tyrosine was tested by the method of Gordon & Smith (1955). Growth temperatures were tested on trypticase soy agar (TSA) at 10, 25, 30, 37 and 45 °C. Tolerance of salt was examined in trypticase soy broth (TSB) containing NaCl at 0–9.0 % (w/v). On TSA, colonies of strain Y2^T were up to 6 mm in diameter and cream-coloured with undulate edges. Aggregates or pellicles were not formed in TSB. Strain Y2^T was aerobic, Gram-positive and slightly acid-alcohol-fast. Detailed physiological properties of strain Y2^T are provided in the species description. Some characteristics that differentiate strain Y2^T from recognized species of the genus *Tsukamurella* are shown in Table 1.

PCR amplification of the 16S rRNA gene was performed with primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3') on a Master gradient PCR machine (Eppendorf) as described by Lane (1991). The amplified DNA fragments were purified and sequenced. Phylogenetic analysis was performed by using the MEGA program, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). 16S rRNA gene sequence studies indicated that strain Y2^T was related to members of the genus *Tsukamurella* (Fig. 1). Sequence similarity calculations after neighbour-joining analysis indicated that the closest relative of strain Y2^T was *T. tyrosinosolvens* (GenBank accession no. AY238514; 99.8 %) (Fig. 1).

Whole-cell fatty acid methyl ester analysis and mycolic acid analyses were performed by Microbial ID, Inc., using the Sherlock Microbial Identification System software (MIDI Inc.). Cellular fatty acids were extracted and analysed by following the instructions and standard procedures of Microbial ID, Inc. (Miller, 1982; Sasser, 1990). The diaminopimelic acid composition and whole cell sugar pattern of strain Y2^T was determined according to

Table 1. Phenotypic differences between strain Y2^T and other closely related species of the genus *Tsukamurella*

Strains: 1, Y2^T; 2, *T. tyrosinosolvens* DSM 44344^T; 3, *T. pulmonis* IMMIB D-1321^T; 4, *T. strandjordii* ATCC BAA-173^T. All colonies were white/cream. All strains were able to grow on (+)-D-arabitol, (+)-D-galactose, citrate, (+)-D-glucose, *p*-hydroxybenzoate, isoamyl alcohol, (+)-D-mannose, (+)-D-melibiose, α -L-rhamnose, (+)-D-salicin, (+)-D-sucrose and (+)-D-trehalose and were able to hydrolyse Tween 80. None of the strains were able to grow on *m*-hydroxybenzoate and did not hydrolyse adenine, gelatin, guanine, hippuric acid, keratin, starch or testosterone. All strains were able to grow at 25 °C and 37 °C, but not at 10 °C or 45 °C. +, Positive or present; –, negative or absent.

Characteristic	1	2	3	4
Growth on sole carbon sources:				
(+)-D-Arabinose	+	+	+	–
(+)-L-Arabinose	+	+	+	–
Benzoate	–	+	–	–
(+)-D-Cellobiose	+	+	+	–
Dulcitol	+	+	+	–
<i>meso</i> -Erythritol	+	+	+	–
(+)-D-Fructose	+	+	+	–
(+)-D-Maltose	+	+	+	–
(–)-D-Mannitol	+	+	–	+
(+)-D-Melezitose	+	+	–	–
(–)-D-Ribose	+	+	+	–
(–)-D-Sorbitol	+	+	–	+
(+)-D-Xylose	+	+	+	–
Hydrolysis of:				
Aesculin	–	+	+	–
Casein	+	–	–	–
Hypoxanthine	+	+	+	–
Tyrosine	+	+	–	–
Urea	+	–	+	+
Xanthine	+	–	–	–

Sonesson *et al.* (1988) and Goodfellow & O'Donnell (1989), respectively, by the Korean Culture Center of Microorganisms (KCCM), Seoul, Korea. The most abundant fatty acids found in strain Y2^T were C_{18:1}ω9c (42.9 %) and C_{16:0} (31.2 %). Analysis of fatty acid profiles also revealed the presence of a small amount of summed feature 1 (C_{16:1}ω6c/C_{16:1}ω7c or C_{15:0} iso 2-OH; 8.5 %), C_{14:0} (3.7 %), tuberculostearic acid (C_{18:0} 10-methyl; 3.7 %), C_{20:1}ω9c (3.6 %), C_{18:0} (1.5 %), C_{17:0} (1.2 %), C_{20:1}ω7c (1.2 %), C_{17:1}ω8c (0.7 %), C_{20:0} (0.7 %), summed feature 6 (C_{19:1}ω9c or C_{19:1}ω11c; 0.5 %), C_{12:0} (0.4 %) and C_{16:1}ω9c (0.3 %) (a comparison of the fatty acid profile of strain Y2^T with those of other species of the genus *Tsukamurella* is available as Supplementary Table S1 in IJSEM Online). Strain Y2^T contained mycolic acids with 81–95 carbon atoms; this is different from the typical number of carbon atoms (64–78 carbon atoms; Collins *et al.*, 1988) usually found for members of the genus *Tsukamurella*. Strain Y2^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell-wall

Fig. 1. Evolutionary distance dendrogram based on nearly complete 16S rRNA gene sequences displaying the relationships between strain Y2^T and representative strains of recognized members of the genus *Tsukamurella*. The tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) based on a comparison of 1452 unambiguous nucleotide positions. Filled circles (●) indicate nodes with 100% bootstrap support. Bar, 1 substitution per 1000 nucleotides.

peptidoglycan. Galactose and arabinose were the characteristic whole-cell sugars. Thus, strain Y2^T is a cell-wall chemotype IV organism.

DNA–DNA hybridization experiments were performed between strain Y2^T and *T. tyrosinosolvens* DSM 44344^T, *T. pulmonis* IMMIB D-1321^T, *T. strandjordii* KACC 20644^T, *T. pseudospumae* KACC 20642^T, *T. spumae* KACC 20641^T, *T. inchonensis* KACC 20573^T and *T. paurometabola* KACC 20551^T using the method described by Nicholas *et al.* (2002). DNA–DNA hybridization showed that the strain Y2^T exhibited a low level of DNA–DNA relatedness with its phylogenetic neighbours *T. tyrosinosolvens*, *T. pulmonis*, *T. strandjordii*, *T. pseudospumae*, *T. spumae*, *T. inchonensis* and *T. paurometabola* (62.7, 60.6, 7.9, 58.7, 12.7, 8.6 and 11.9 %, respectively; mean value of three hybridizations; SD, 3.4, 5.0, 2.4, 2.5, 2.5, 0.7 and 3.2 %, respectively). The DNA base composition (G+C mol%) was determined according to Mesbah *et al.* (1989) by the KCCM. The G+C content of strain Y2^T was 77 mol% and the DNA G+C content of recognized species of the genus ranges from 69 to 74 mol% (Yassin *et al.*, 1997).

On the basis of 16S rRNA gene sequence and whole-cell fatty acid methyl ester analyses, strain Y2^T was placed within the genus *Tsukamurella*. Strain Y2^T could be easily differentiated from *T. pseudospumae*, *T. spumae*, *T. inchonensis* and *T. paurometabola* by 16S rRNA sequence-based phylogenetic analyses and by DNA–DNA hybridization. Strain Y2^T was also

clearly distinguishable from *T. tyrosinosolvens*, *T. pulmonis* and *T. strandjordii* based on its ability to decompose and hydrolyse several chemicals, together with the phylogenetic and DNA–DNA hybridization data. Furthermore, the strain Y2^T contained mycolic acids with longer carbon chains and DNA with a higher G+C content than those of other species of the genus *Tsukamurella*. Thus it is reasonable to assign strain Y2^T as a novel species of the genus *Tsukamurella*, for which the name *Tsukamurella carboxydivorans* is proposed.

Description of *Tsukamurella carboxydivorans* sp. nov.

Tsukamurella carboxydivorans (car.bo.xy.di.vor'ans. N.L. neut. n. *carboxydum* carbon monoxide; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *carboxydivorans* digesting carbon monoxide).

Aerobic, Gram-positive, slightly acid–alcohol-fast, non-motile actinomycete. Grows optimally at 30 °C and tolerates up to 6 % (w/v) NaCl. Positive in tests for oxidase. Is able to utilize the following as sole carbon sources: (+)-D-arabinose, (+)-L-arabinose, (+)-D-cellobiose, dulcitol, *meso*-erythritol, (+)-D-fructose, (+)-D-maltose, (–)-D-mannitol, (+)-D-melezitose, (–)-D-ribose, (–)-D-sorbitol and (+)-D-xylose. Hydrolyses casein, hypoxanthine, tyrosine and urea, but does not hydrolyse aesculin. Predominant fatty acids are C_{18:1ω9c} and C_{16:0}. Contains mycolic acids with 81–95 carbon atoms. The

diagnostic diamino acid in the cell-wall peptidoglycan is *meso*-diaminopimelic acid. Galactose and arabinose are the whole cell sugars (cell-wall chemotype IV).

The type strain, Y2^T (=KCCM 42885^T=JCM 15482^T), was isolated from subsurface soil samples (10 cm depth) collected from the side of a busy road at Yonsei University, Seoul, Korea. The DNA G+C content of the type strain is 77 mol%.

Acknowledgements

This work was supported by a research grant (F01-2003-000-0004-0) from the Korea Science and Engineering Foundation.

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