**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 Y2T, was isolated from a soil sample collected from a roadside in Seoul, Korea. On the basis of 16S rRNA gene sequence comparative analyses, strain Y2T was shown to belong to the genus *Tsukamurella* and was most closely related to *Tsukamurella tyrosinosolvens* DSM 44234T (GenBank accession no. AY238514; 99.8%). The predominant fatty acids were C18:1ω9c and C16:0. The cell-wall peptidoglycan of strain Y2T contained meso-diaminopimelic acid as the diagnostic diamino acid. Strain Y2T contained galactose and arabinose as the whole cell sugars. The DNA G+C content was 77 mol%. The DNA–DNA relatedness value between strain Y2T and *T. tyrosinosolvens* DSM 44234T 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 Y2T (=KCCM 42885T=JCM 15482T) 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 inchoenensis* (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 Y2T, 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 Y2T 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 photometrically by measuring the CO-dependent reduction of 2-(4-indophenyl)-3-(4-nitrophenyl)-2H-tetrazolium chloride (INT, ε490=17.981 mM–1 cm–1) (Kraut *et al.*, 1989). Crude extracts prepared from cells of strain Y2T 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 Y2T was isolated by using cetyletrimethylammonium bromide as described by Ausubel...
et al. (1995). The partial CO-DH large subunit gene was amplified with primers BMSF (5'-GGCGGCTTYGGSTCSAAGAT-3') and O/Br (5'-TYCGAYGATCATCGGRTGA-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 Y2T 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, ascuscin 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 Y2T were up to 6 mm in diameter and cream-coloured with undulate edges. Aggregates or pellicles were not formed in TSB. Strain Y2T was aerobic, Gram-positive and slightly acid–alcohol-fast. Detailed physiological properties of strain Y2T are provided in the species description. Some characteristics that differentiate strain Y2T 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'-AAGAGGAGGTGWTCCARCC-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 Y2T was related to members of the genus Tsukamurella (Fig. 1). Sequence similarity calculations after neighbour-joining analysis indicated that the closest relative of strain Y2T was T. tyrosinosolvens (GenBank accession no. AJ238514; 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 Y2T was determined according to

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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 Y2T were C18:1ω9c (42.9 %) and C16:0 (31.2 %). Analysis of fatty acid profiles also revealed the presence of a small amount of summed feature 1 (C16:1ω6c/C16:1ω7c or C15:0 iso 2-OH; 8.5 %), C14:0 (3.7 %), tuberculostearic acid (C18:0 10-methyl; 3.7 %), C20:1ω9c (3.6 %), C18:0 (1.5 %), C17:0 (1.2 %), C20:1ω7c (1.2 %), C17:0ω8c (0.7 %), C20:0 (0.7 %), summed feature 6 (C19:1ω9c or C19:1ω11c; 0.5 %), C12:0 (0.4 %) and C16:1ω7c (0.3 %) (a comparison of the fatty acid profile of strain Y2T with those of other species of the genus Tsukamurella is available as Supplementary Table S1 in IJSEM Online). Strain Y2T contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell-wall.
peptidoglycan. Galactose and arabinose were the characteristic whole-cell sugars. Thus, strain Y2T is a cell-wall chemotype IV organism.

DNA–DNA hybridization experiments were performed between strain Y2T and *T. tyrosinosolvens* DSM 44344T, *T. pulmonis* IMMIB D-1321T, *T. strandjordii* KACC 20644T, *T. pseudospumae* KACC 20641T, *T. inchonensis* KACC 20573T and *T. paurometabola* KACC 20551T using the method described by Nicholas et al. (2002). DNA–DNA hybridization showed that the strain Y2T 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, 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 Y2T was 77 mol% and the DNA G+C content of recognized species of the genus *Tsukamurella* 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 Y2T was placed within the genus *Tsukamurella*. Strain Y2T 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 Y2T 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 Y2T 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 Y2T as a novel species of the genus *Tsukamurella*, for which the name *Tsukamurella carboxydivorans* is proposed.

**Description of *Tsukamurella carboxydivorans* sp. nov.**


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-cellulbiose, dulcitol, *meso*-erythritol, (+)-D-fructose, (+)-D-maltose, (+)-D-mannitol, (+)-D-melezitose, (+)-D-ribose, (+)-D-sorbitol and (+)-D-xyllose. Hydrolyses casein, hypoxanthine, tyrosine and urea, but does not hydrolyse aesculin. Predominant fatty acids are C<sub>18:1</sub>ω9c and C<sub>16:0</sub>. Contains mycolic acids with 81–95 carbon atoms. The

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Fig. 1. Evolutionary distance dendrogram based on nearly complete 16S rRNA gene sequences displaying the relationships between strain Y2T 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.
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, Y2T (KCCM 42885T=JCM 15482T), 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

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References


