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Tsukamurella pseudospumae sp. nov., a novel actinomycete isolated from activated sludge foam

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The taxonomic position of two *Tsukamurella* strains isolated from activated sludge foam was clarified. The organisms, isolates JC85 and N1176^T, were found to have chemical and morphological properties typical of members of the genus *Tsukamurella*. DNA–DNA relatedness studies showed that the strains formed a distinct genomic species that was most closely related to *Tsukamurella spumae*. The two isolates also share a range of phenotypic properties that distinguishes them from representatives of all species of *Tsukamurella* with validly published names. It is evident from the data that the two organisms should be classified as a novel *Tsukamurella* species, *Tsukamurella pseudospumae* sp. nov. The type strain is N1176^T (=DSM 44118^T=NCIMB 13963^T).

The monospecific genus *Tsukamurella* was proposed by Collins *et al.* (1988) for organisms previously classified as *Corynebacterium paurometabolum* (Steinhaus, 1941) and '*Gordona aurantiaca*' (Tsukamura & Mizuno, 1971). The taxon is well defined and currently encompasses six species with validly published names, namely *Tsukamurella inchonensis* Yassin *et al.* 1995, *Tsukamurella pulmonis* Yassin *et al.* 1996, *Tsukamurella paurometabola* (Steinhaus 1941) Collins *et al.* 1988, *Tsukamurella spumae* Nam *et al.* 2003, *Tsukamurella strandjordii* Kattar *et al.* 2001 and *Tsukamurella tyrosinosolvens* Yassin *et al.* 1997. These species form a distinct clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes, i.e. by organisms classified in the suborder *Corynebacterineae* Stackebrandt *et al.* 1997. Members of these taxa share very high 16S rRNA gene nucleotide similarity values but can be distinguished by DNA–DNA relatedness and phenotypic data (Kattar *et al.*, 2001; Nam *et al.*, 2003). Tsukamurellae have been described in association with clinical disease (Yassin *et al.*, 1995, 1996, 1997; Kattar *et al.*, 2001) and as agents of foaming in activated sludge plants (Goodfellow *et al.*, 1996, 1998; Seong *et al.*, 1999). Activated sludge foams cause operational problems and may represent a public health hazard because of the potential spread of pathogens by aerosols (Goodfellow *et al.*, 1998).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *T. pseudospumae* strains N1176^T and JC85 are AY238513 and AY333425.

The aim of the present study was to establish the taxonomic position of two organisms, represented by strains JC85 and N1176^T, isolated from activated sludge foam and provisionally labelled '*Tsukamurella spumae*' (Goodfellow *et al.*, 1998). It was evident from this study that the two isolates were closely related on the basis of whole-organism pyrolysis mass spectrometric data. In a recent polyphasic study, strain N1176^T was distinguished from strains of *T. spumae* and considered to represent a prospective novel species of the genus *Tsukamurella* (Nam *et al.*, 2003).

The two organisms were maintained as glycerol suspensions (20 %, v/v) at −20 °C and as glucose/yeast extract agar slopes (Gordon & Mihm, 1962) at room temperature, as were the type strains of *Tsukamurella* species (Table 1). Strain JC85 was examined for a combination of phenotypic tests, using standard procedures (Chun, 1995; Nam *et al.*, 2003). Extraction of chromosomal DNA, PCR amplification and the isolation, cloning and sequencing of the amplified 16S rDNA of strain JC85 was carried out following established procedures (Chun & Goodfellow, 1995). The resultant 16S rRNA gene sequence was compared to corresponding sequences of representatives of the suborder *Corynebacterineae* as described previously (Nam *et al.*, 2003).

The extent of DNA–DNA relatedness between isolate N1176^T, isolate JC85 and the type strains of *Tsukamurella* species was estimated using the fluorometric microplate method (Ezaki *et al.*, 1989), as modified by Goris *et al.*

Table 1. *Tsukamurella* strains included in this study

Strain(s)	Isolated by	Source
T. inchonensis N1238^T (=IMMIB D-771^T)	A. F. Yassin, Institute of Medical Microbiology and Immunology, University of Bonn, Bonn, Germany	Blood cultures of a patient who had ingested hydrochloric acid
T. paurometabola JC7^T (=DSM 20162^T= ATCC 8368^T)	E. A. Steinhaus (*Corynebacterium paurometabolum*)	Mycetomes and ovaries of the bed bug (*Cimex lectularus*)
T. pulmonis N1240^T (=IMMIB D-1321^T)	A. F. Yassin	Sputum from a patient with pulmonary tuberculosis
T. spumae N1171^T, N1173	J. Chun	Activated sludge foam, Stoke Bardolph Water Reclamation Works near Nottingham, UK
T. pseudospumae N1176^T	J. Chun	Activated sludge foam, Stoke Bardolph Water Reclamation Works
T. pseudospumae JC85	F. M. Stainsby, School of Biology, University of Newcastle, Newcastle upon Tyne, UK	Activated sludge foam, Stoke Bardolph Water Reclamation Works
T. strandjordii N1275^T	L. C. Carlson, Department of Laboratory Medicine, University of Washington, Seattle, WA, USA	Blood from a 5-year-old girl with acute mycelogenous leukaemia
T. tyrosinosolvens N1274^T (=IMMIB D-1397^T)	A. F. Yassin	Blood culture of a patient with a cardiac pacemaker implant

(1998). Photobiotin-labelled DNA from isolate N1176^T was individually hybridized with single-stranded unlabelled DNA samples of the remaining strains, non-covalently bound to microtitre wells. The hybridization experiments were conducted under stringent conditions in 50 % formamide at 43 °C. Fluorescent intensities were measured using a Fluoroskan CF fluorimeter (Thermo Lab Systems Inc.) at a wavelength of 360 mm for excitation and 450 mm for emission. Mean percentage DNA–DNA relatedness values were calculated from triplicate hybridization experiments.

The morphological, degradative, nutritional and physiological properties recorded for isolate JC85 were virtually identical to those reported for strain N1176^T (Nam *et al.*, 2003), a result that underpins the close relationship found between these organisms based on Curie-point pyrolysis mass spectrometry (Goodfellow *et al.*, 1998). Strain JC85 was also found to contain *meso*-A_2pm, arabinose and galactose in whole-organism hydrolysates (wall chemotype IV *sensu* Lechevalier *et al.*, 1971), *N*-glycolyl residues in the glycan moiety of the cell wall, unsaturated menaquinones with nine isoprene units as the predominant isoprenologue, mycolic acids and major proportions of hexadecanoic (23·3 % of total fatty acids), oleic (29·9 %) and tuberculostearic (15·2 %) acids. All of these chemical properties are consistent with the classification of strain JC85 in the genus *Tsukamurella* (Collins *et al.*, 1988; Yassin *et al.*, 1995, 1996, 1997; Kattar *et al.*, 2001).

It is evident from Fig. 1 that strains JC85 and N1176^T have identical 16S rRNA gene sequences. The mean DNA–DNA relatedness values and standard deviations found between strain N1176^T and *T. inchonensis* N1238^T, *T. paurometabola* JC7^T, *T. pulmonis* N1240^T, *T. strandjordii* N1275^T, *T. tyrosinosolvens* N1274^T and *T. spumae* N1173 were $35\pm0{\cdot}6$, $15\pm0{\cdot}8$, $42\pm0{\cdot}7$, $34\pm1{\cdot}1$, $35\pm0{\cdot}7$ and $46\pm0{\cdot}7$ %, respectively, values well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for the

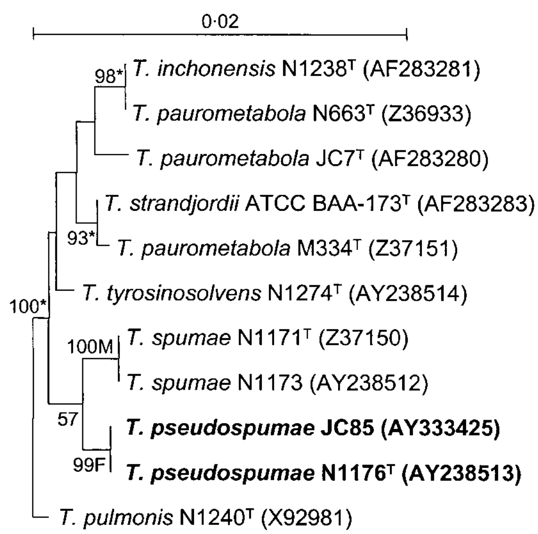

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing relationships between strains JC85 and N1176^T and representative strains of *Tsukamurella* species. Branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967) and/or maximum-likelihood (Felsenstein, 1981) treeing algorithms are indicated by asterisks (both algorithms) or by F (least-squares only) and M (maximum-likelihood only). Numbers at nodes indicate the level of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0·2 substitutions per nucleotide position.

Table 2. Phenotypic properties separating strains JC85 and N1176^{T} from the type strains of *Tsukamurella* species

Taxa are identified as: 1, strains JC85 and N1176^{T}; 2, *Tsukamurella inchonensis* N1238^{T}; 3, *Tsukamurella paurometabola* JC7^{T}; 4, *Tsukamurella pulmonis* N1240^{T}; 5, *Tsukamurella spumae* N1171^{T}; 6, *Tsukamurella strandjordii* N1275^{T}; 6, *Tsukamurella tyrosinosolvens* N1274^{T}. Data for all of the recognized species are taken from Nam *et al.* (2003). Characteristics are scored as: +, positive; −, negative.

Characteristic	1	2	3	4	5	6	7
Biochemical tests:							
Aesculin hydrolysis	+	+	+	+	−	+	+
Urea hydrolysis	−	+	+	+	−	+	−
Colour of colonies:							
Orange/red	+	−	−	−	+	−	−
White/cream	−	+	+	+	−	+	+
Degradation tests:							
Hypoxanthine	+	+	−	+	+	−	+
Tyrosine	+	−	−	−	+	−	+
Growth at 10 °C	+	−	+	−	−	−	−
Growth on sole carbon sources (1 %, w/v):							
D(+)-Arabinose	+	+	−	+	−	−	+
L(+)-Arabinose	+	−	−	+	+	−	+
D(+)-Arabitol	+	−	−	+	+	+	+
D(+)-Cellobiose	−	−	−	+	−	−	+
Dulcitol	−	−	−	+	+	−	+
meso-Erythritol	−	−	−	+	+	−	+
D(+)-Fructose	+	−	+	+	+	−	+
D(+)-Maltose	+	+	−	+	+	−	+
D(−)-Mannitol	−	+	+	−	+	+	+
D(+)-Melezitose	+	+	−	−	+	−	+
D(+)-Melibiose	−	+	+	+	+	+	+
D(−)-Ribose	+	+	+	+	+	−	+
D(+)-Salicin	−	+	+	+	−	+	+
D(−)-Sorbitol	−	+	−	−	+	+	+
D(+)-Xylose	−	+	+	+	+	−	+

delineation of genomic species. In contrast, strains N1176^{T} and JC85 shared a mean DNA–DNA relatedness value of 82 ± 2·0 % and hence are considered to belong to the same genomic species. Strain JC85 shared mean DNA–DNA relatedness values with *T. inchonensis* N663, *T. paurometabola* JC7^{T} and *T. spumae* N1173 of 22 ± 0·1, 14 ± 0·4 and 58 ± 1·4 %, respectively. It can be seen from Table 2 that strains JC85 and N1176^{T} share several phenotypic properties that readily distinguish them from representatives of *Tsukamurella* species with validly published names.

It is apparent from this and earlier studies that strains JC85 and N1176^{T} belong to a single species that can be separated from representatives of all species of *Tsukamurella* using a combination of genotypic and phenotypic data. It is proposed that these organisms be classified in the genus *Tsukamurella* as *Tsukamurella pseudospumae* sp. nov.

Description of *Tsukamurella pseudospumae* sp. nov.

Tsukamurella pseudospumae (pseu.do.spu′mae. Gr. adj. *pseudes* false; L. gen. n. *spumae* of foam and specific epithet of a bacterial species; N.L. n. *pseudospumae* the false *spumae*, referring to the close relationship to *Tsukamurella spumae*).

The description is based on data taken from this and earlier studies (Chun, 1995; Goodfellow *et al.*, 1998; Nam *et al.*, 2003). Aerobic, Gram-positive, partially acid–alcohol-fast, non-motile, non-spore-forming actinomycete, which forms straight to slightly curved rods and a few long filaments, but which does not differentiate into substrate or aerial hyphae. Colonies on glucose/yeast extract agar are large (<5 mm), orange to red with irregular margins and elevation. Tween 80 and uric acid are degraded but xanthine and xylan are not. Grows at 10, 25 and 37 °C, but not at 45 °C. D(+)-Galactose, D(−)-gentiobiose, D(+)-glucose, *meso*-inositol, D(−)-lactose, D(+)-mannose, α-L-rhamnose, D(+)-sucrose, D(+)-trehalose, D(+)-turanose, D(−)-xylitol (1 %, w/v), amyl alcohol, butane-2,3-diol (1 %, v/v), sodium citrate and sodium pyruvate (0·1 %, w/v) are used as sole sources of carbon for energy and growth, but adonitol (1 %, w/v), butane-1,4-diol, ethanolamine, D(−)-glucuronic acid, methanol (1 %, v/v), resorcinol, sodium benzoate and sodium tartrate (0·1 %, w/v) are not. L-Asparagine, L-phenylalanine and L-serine are used as sole carbon and nitrogen sources, but L-histidine, L-lysine, succinamide and L-valine are not. Resistant to crystal violet (0·001 %, w/v), 5-fluorouracil (20 μg ml^{-1}), bekanamycin (16, 32 and 64 μg ml^{-1}), clindamycin (2 μg ml^{-1}), colistin (25 μg ml^{-1}), fusidic acid (10 μg ml^{-1}), gentamicin sulphate (16 and 32 μg ml^{-1}), kanamycin sulphate (4, 8, 16 and 32 μg ml^{-1}), nalidixic acid (5 μg ml^{-1}), neomycin sulphate (4, 8, 16 and 32 μg ml^{-1}), novobiocin (16 μg ml^{-1}), oleandomycin phosphate (16, 32 and 64 μg ml^{-1}), rifampicin (0·5 and 2 μg ml^{-1}), tetracycline hydrochloride (10 μg ml^{-1}) and vancomycin hydrochloride (1, 2 and 4 μg ml^{-1}), but susceptible to chlortetracycline hydrochloride (2 and 8 μg ml^{-1}), ciprofloxacin (5 μg ml^{-1}), erythromycin (2, 4 and 8 μg ml^{-1}), novobiocin (64 μg ml^{-1}), penicillin G (16, 32 and 64 μg ml^{-1}) and rifampicin (8 and 16 μg ml^{-1}). Other phenotypic properties are shown in Table 2. Contains mycolic acids with 68–76 carbon atoms and up to seven double bonds; the major products from pyrolysis gas chromatography of methyl mycolates are straight-chain fatty acids $C_{20:1}$ and $C_{22:1}$. Additional chemical markers are typical of members of the genus *Tsukamurella*.

The type strain is N1176^{T} (=DSM 44118^{T}=NCIMB 13963^{T}). Strains JC85 and N1176^{T} were isolated from activated sludge foam collected from Stoke Bardolph Water Reclamation Works near Nottingham, UK.

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