

Streptomyces karpasiensis sp. nov., isolated from soil

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A novel actinobacteria, designated strain K413^T, was isolated from soil collected from Karpaz National Park, Magusa, Northern Cyprus, and characterized to determine its taxonomic position. The isolate was found to have chemical and morphological properties associated with members of the genus *Streptomyces*. Phylogenetic analyses based on almost-complete 16S rRNA gene sequences indicated that the isolate was closely related to members of the genus *Streptomyces*, and was shown to form a distinct phyletic line in the *Streptomyces* phylogenetic tree. Strain K413^T was most closely related to *Streptomyces marinus* DSM 41968^T (98.01 %). Sequence similarities with other strains of the genus *Streptomyces* were below 98.0 %. The cell wall of the novel strain contained LL-diaminopimelic acid. The predominant menaquinone was MK-9(H₈) (45.0 %). The polar lipids detected were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and phosphatidylinositol. The major fatty acids were anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. Based on 16S rRNA gene sequence analysis, DNA–DNA relatedness, phenotypic characteristics and chemotaxonomic data, strain K413^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces karpasiensis* sp. nov. is proposed. The type strain is K413^T (=KCTC 29096^T=DSM 42068^T).

The genus *Streptomyces*, proposed by Waksman & Henrici (1943), contains the largest number of species of any genus in the domain *Bacteria*, with over 600 species with validly published names at the time of writing (<http://www.bacterio.cict.fr/s/streptomycetes.html>). Members of this genus are well-known as a rich source of bioactive secondary metabolites, such as antibiotics and industrially and commercially important enzymes, hence the continued interest in screening for novel streptomycetes (Chater *et al.*, 2010; Goodfellow & Fiedler, 2010; Rateb *et al.*, 2011; Kim *et al.*, 2012a; Santhanam *et al.*, 2013). The cost of discovering novel secondary metabolites from common species of the genus *Streptomyces* is making it increasingly counterproductive. Hence, there is a need to isolate and describe streptomycetes from diverse environments to screen for novel bioactive compounds (Busti *et al.*, 2006; Lam, 2007; Goodfellow & Fiedler, 2010;

Santhanam *et al.*, 2013). In our continuing research on culturable actinobacterial biodiversity from diverse habitats, a putative novel strain of the genus *Streptomyces*, K413^T, was isolated from a soil sample from Karpaz National Park, Magusa, Northern Cyprus. The present study was undertaken to establish the taxonomic position of isolate K413^T using a polyphasic approach.

Strain K413^T was isolated from a soil sample collected from Karpaz National Park, Magusa, Northern Cyprus, after incubation for 2 weeks at 28 °C on humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987), supplemented with cycloheximide (50 µg ml⁻¹) and nalidixic acid (10 µg ml⁻¹). The strain was isolated as a pure culture, maintained on yeast malt extract agar [International *Streptomyces* Project medium 2 (ISP 2); Shirling & Gottlieb, 1966] slopes at room temperature and stored as glycerol suspensions (20 %, v/v) at –20 °C.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product were carried out according to methods described by Chun & Goodfellow (1995). The almost-complete (1468 bp) 16S

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain K413^T is JQ864430.

A supplementary figure and a supplementary table are available with the online version of this paper.

rRNA gene sequence of strain K413^T was determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net>; Kim *et al.*, 2012b). Multiple alignment with sequences from closely related species was performed using the program CLUSTAL W in the MEGA5 software package (Tamura *et al.*, 2011). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA5 (Tamura *et al.*, 2011). Evolutionary distances were calculated using the model of Jukes & Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings.

The 1468 bp sequence corresponding to the 16S rRNA gene region of strain K413^T was compared with sequences deposited in public databases. Comparison of the almost-complete 16S rRNA gene sequence of the isolate with corresponding sequences of phylogenetically related species with validly published names showed that it formed a new phyletic line at the periphery of the *Streptomyces marinus* DSM 41968^T 16S rRNA gene tree, a result supported by all the tree-making algorithms used and by a bootstrap value of 87 % (Fig. 1). Strain K413^T shares 98.01 % 16S rRNA gene similarity with *S. marinus* DSM 41968^T, a value that corresponds to 29 nt differences at 1457 sites. The highest 16S rRNA sequence similarities between strain K413^T and type strains of recognized species in the databases were 97.75 % (33 nt differences at 1468 sites) to *Streptomyces glycovorans* YIM M 10366^T, 97.68 % (34 nt differences at 1464 sites) to *Streptomyces haliclonae* DSM 41970^T, 97.55 % (36 nt differences at 1468 sites) to *Streptomyces xishensis* YIM M 10378^T, 97.34 % (39 nt differences at 1468 sites) to *Streptomyces qinglanensis* 172205^T, 97.34 % (39 nt differences at 1467 sites) to *Streptomyces panacagri* Gsoil 519^T, 97.14 % (42 nt differences at 1468 sites) to *Streptomyces albus* subsp. *albus* NRRL B-2365, 97.13 % (42 nt differences at 1464 sites) to *Streptomyces rangoonensis* LMG 20295^T, 97.13 % (42 nt differences at 1463 sites) to *Streptomyces gibsonii* NBRC 15415^T, 97.12 % (42 nt differences at 1458 sites) to *Streptomyces almqvistii* NBRC 13015^T. Sequence similarities with strains of other species of the genus *Streptomyces* were lower than 97.0 %.

DNA–DNA hybridization experiments were performed with strain K413^T and type strains of the most closely related species, *S. marinus* DSM 41968^T, *S. glycovorans* YIM M 10366^T, *S. xishensis* YIM M 10378^T and *S. haliclonae* DSM 41970^T, by the Identification Service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), incorporating the modifications described by Huß *et al.* (1983), using a model

Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

The taxonomic integrity of the test strains was supported by DNA–DNA relatedness data. Strain K413^T showed DNA–DNA relatedness values of 45.8 % ± 2.5 % to *S. marinus* DSM 41968^T, 40.1 % ± 3.6 % to *S. glycovorans* YIM M 10366^T, 37.4 % ± 1.8 % to *S. haliclonae* DSM 41970^T and 30.2 % ± 4.1 % to *S. xishensis* YIM M 10378^T (based on a mean of duplicate determinations in 2 × SSC and 10 % formamide at 70 °C); all values were below the recommended criterion of 80 % for delineation of species of the genus *Streptomyces* (Labeda, 1992).

Chemotaxonomic analyses were carried out to support the phylogenetic affiliation of strain K413^T to the genus *Streptomyces*. The strain was grown in GYM broth (DSMZ Medium 65) under aerobic conditions in flasks on a rotary shaker at 160 r.p.m. and 28 °C for 10 days. Biomass was harvested by centrifugation, washed twice in distilled water, recentrifuged and freeze-dried. Gram-staining was performed by the non-staining method described by Buck (1982). Isomers of diaminopimelic acid in whole-cell hydrolysates and sugars were prepared according to Lechevalier & Lechevalier (1970) and analysed by thin-layer chromatography (Staneck & Roberts, 1974). Polar lipid and respiratory quinone analyses were carried out by the Identification Service of the DSMZ. Respiratory quinones were extracted from 100 mg freeze-dried cells based on the two-stage method described by Tindall (1990a, 1990b). Respiratory quinones were separated into their different classes (menaquinones and ubiquinones) by thin-layer chromatography on silica gel (Macherey-Nagel Art. no. 805 023), using hexane/tert-butylmethylether (9 : 1, v/v) as the solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on a LDC Analytical HPLC (Thermo Separation Products) fitted with a reverse phase column (Macherey-Nagel, 2 mm × 125 mm, 3 µm, RP18) using methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

A starter culture for the fatty acid analyses was prepared in a flask containing 20 ml N-Z-amine broth (DSM medium 554), which was shaken at 160 r.p.m. at 28 °C for 5 days. Five millilitres of the resultant culture was used to inoculate 50 ml of N-Z-amine, which was incubated under the same conditions. Biomass was harvested by cellulose filtration (pore size 0.45 µm) and the wet cells (200 mg) were placed in an extraction tube. Cellular fatty acids were extracted, methylated and separated by gas chromatography using an Agilent Technologies 6890 N instrument, fitted with an autosampler and a 6783 injector, according to the standard protocol of the MIDI Sherlock Microbial Identification System (Sasser, 1990; Kämpfer & Kroppenstedt, 1996); fatty acid methyl ester peaks were quantified using TSBA 5.0 software. The DNA G + C content of the isolate

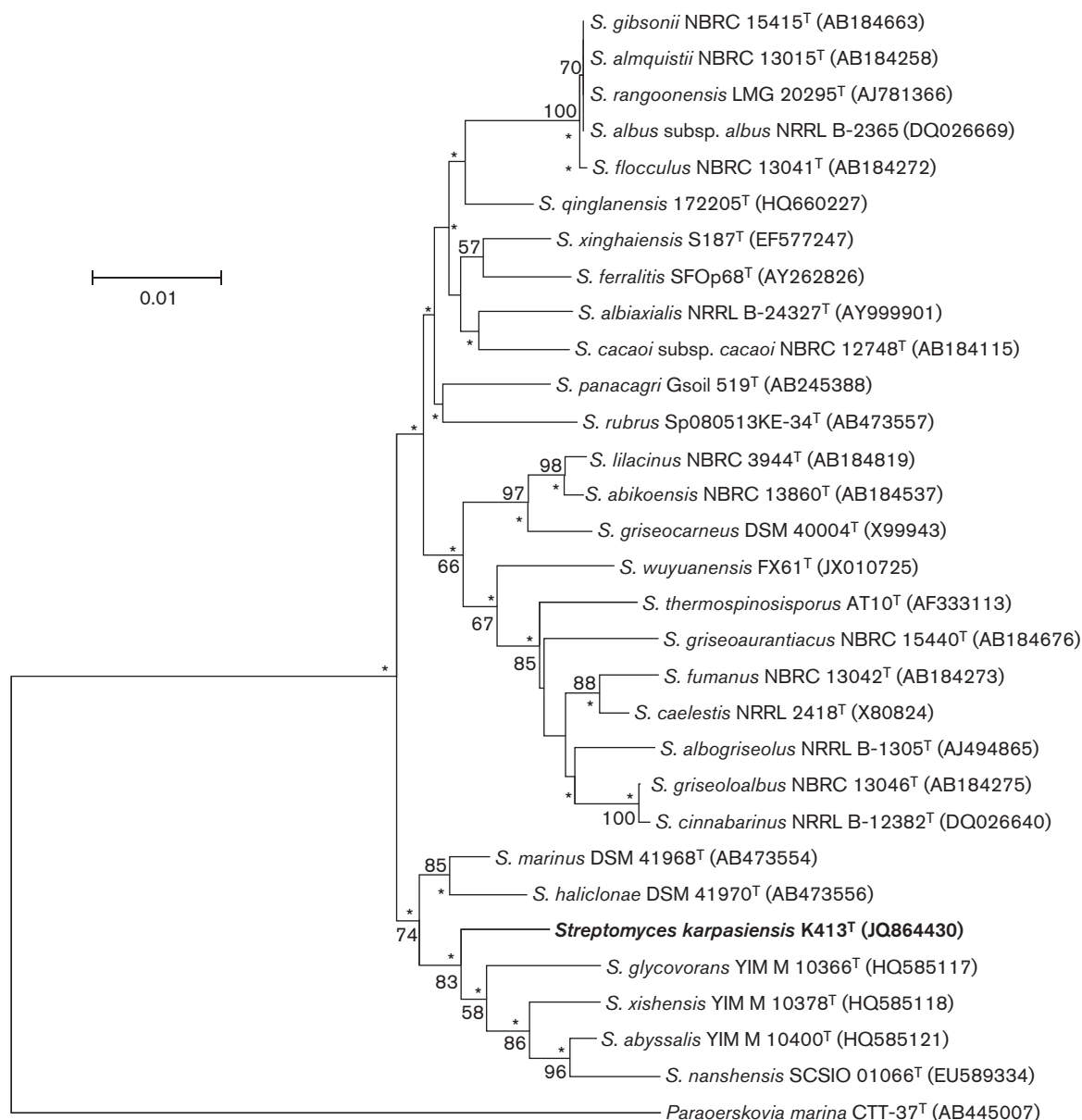


Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the position of strain K413^T amongst its phylogenetic neighbours. Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. *Paraoerskovia marina* CTT-37^T (GenBank accession no. AB445007) was used as an outgroup. Numbers at the nodes indicate the levels of bootstrap support (%); only values $\geq 50\%$ are shown. Numbers in parentheses are GenBank accession numbers. Bar, 0.01 substitutions per site.

was determined by following the procedure of Gonzalez & Saiz-Jimenez (2005).

The cell-wall diamino acid of strain K413^T was LL-diaminopimelic acid and glycine (Type I; Lechevalier & Lechevalier, 1970), and the whole-cell hydrolysates contained mainly galactose and glucose, with small amounts of mannose and ribose. The polar lipids of strain K413^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine,

phosphatidylinositol, three unidentified phospholipids, two unidentified phosphoglycerolipids and two unidentified glycolipids (Fig. S1, available in the online Supplementary Material). The predominant menaquinone of strain K413^T was MK-9(H₈) (45.0%); in addition MK-9(H₆) (6.0%) and MK-9(H₄) (2.0%) and an unknown component with a retention time really close to MK-9(H₈) were also detected. The major cellular fatty acids were anteiso-C_{15:0} (30.15%), iso-C_{16:0} (23.90%) and anteiso-C_{17:0} (14.97%). Comparative

cellular fatty acid compositions of strain K413^T and *S. marinus* DSM 41968^T, *S. glycovorans* YIM M 10336^T, *S. haliclona* DSM 41970^T and *S. xishensis* YIM M 10378^T are shown in Table S1. The G + C content of the DNA of strain K413^T was 71.9 mol%.

Cultural characteristics were investigated on media from the ISP (Shirling & Gottlieb, 1966), modified Bennett's agar (MBA; Jones, 1949), Czapek's and tryptic soy agar (TSA; Difco). The degree of growth, aerial mycelium and pigmentation were recorded after incubation for 14 days at 28 °C. The Inter-Society Color Council–National Bureau of Standards colour charts (Kelly, 1964) were used to designate colour and names. Colony morphology and micro-morphological properties of strain K413^T were determined by examining gold-coated dehydrated specimens of 14-day cultures from ISP 3 (oatmeal) medium using a JEOL JSM 6060 scanning electron microscope. Growth at different temperatures (4, 10, 20, 28, 37, 45 and 50 °C), and pH 4.0–11.0 (at intervals of 1.0 pH unit), and in the presence of 0–10% (w/v) NaCl was determined on ISP 2 medium. Established methods were used to determine whether the strains degraded Tweens 20 and 80 (Nash & Krent, 1991); the remaining degradation tests were carried out using the methods described by Williams *et al.* (1983). Carbon source utilization was tested using carbon source utilization (ISP 9) medium (Shirling & Gottlieb, 1966) supplemented with the carbon sources tested at a final concentration of 1% (w/v). Nitrogen source utilization was examined using the basal medium recommended by Williams *et al.* (1983) supplemented with the nitrogen sources tested at a final concentration of 0.1% (w/v). The type strains *S. marinus* DSM 41968^T, *S. glycovorans* YIM M 10366^T, *S. xishensis* YIM M 10378^T and *S. haliclona* DSM 41970^T were included for comparison in all tests. The commercially available API ZYM system (bioMérieux) was used for the biochemical characterization of strain K413^T, according to the manufacturer's instructions.

Strain K413^T formed a branched white substrate mycelium and white aerial hyphae, which differentiated into spiral chains of smooth-surfaced spores on ISP 3 (oatmeal) medium (Fig. 2). The organism grew well on ISP 3, 4, 5 and 7, MBA and TSA, but grew moderately on ISP 2 and 6, and Czapek's agar. No diffusible pigment was detected on any of the tested media. Melanoid pigments were not produced on ISP 6 or 7 medium. The physiological and biochemical properties of strain K413^T are presented in Table 1 and in the species description.

It is evident that strain K413^T can be distinguished chemotaxonomically and phenotypically from its phylogenetic relatives *S. marinus* DSM 41968^T, *S. glycovorans* YIM M 10366^T, *S. xishensis* YIM M 10378^T and *S. haliclona* DSM 41970^T. Based on 16S rRNA gene sequence analysis, DNA–DNA relatedness, and phenotypic data such as using L-rhamnose and lactose as sole carbon sources, and glycine, L-hydroxyproline and proline as sole nitrogen sources, It is concluded that strain K413^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces karpasiensis* sp. nov. is proposed.

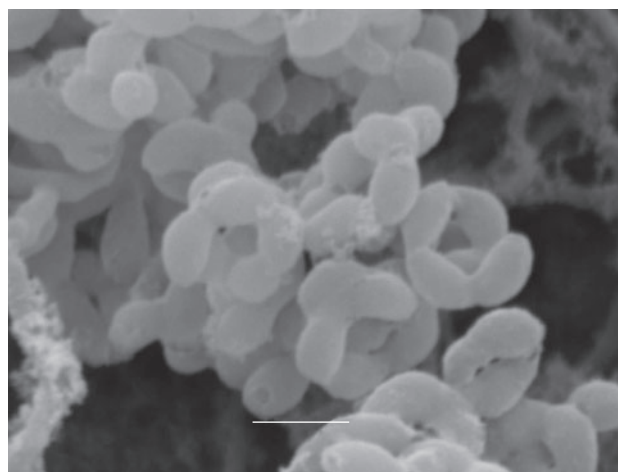


Fig. 2. Scanning electron micrograph of strain K413^T grown on oatmeal medium (ISP 3) at 28 °C for 14 days. Bar, 1 µm.

Description of *Streptomyces karpasiensis* sp. nov.

Streptomyces karpasiensis (kar.pa.si.en'sis. N.L. masc. adj. *karpasiensis* of or belonging to Karpasia, Magusa, Northern Cyprus, the source of the type strain).

Aerobic, Gram-reaction-positive, non-motile, non-acid–alcohol-fast actinomycete that forms branched substrate hyphae and aerial mycelium that differentiate into spiral chains of smooth-surfaced spores. Growth occurs at pH 5.0–10, and at 25–37 °C, but not at pH 4.0 or 11, or at temperatures of 4, 10, 20 and 45 °C. Optimal growth occurs on ISP 2 medium at 28 °C and pH 7.2. Arbutin, allantoin and urea are hydrolysed, but aesculin is not. Nitrate reduction is negative. Adenine, casein, elastin, guanine, hypoxanthine, xanthine, xylan, Tweens 20 and 80 are not degraded. Utilizes L-arabinose, cellobiose, D-galactose, D-mannitol, inulin, L-rhamnose, lactose, starch, and dextrin as sole carbon sources, but not maltose, D-sorbitol or sucrose. Utilizes glycine, L-phenylalanine, L-valine, L-proline and L-hydroxyproline as sole nitrogen sources, but not L-arginine, L-cysteine, L-methionine or L-serine. Positive result in tests for alkaline phosphatase, esterase, esterase-lipase, leucine arylamidase, α-chymotrypsin, α-mannosidase, N-acetyl-β-glucosaminidase and trypsin activities, but negative result for acid phosphatase, α-galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, β-galactosidase, β-glucuronidase, lipase and naphthol-AS-BI-phosphohydrolase activities. The predominant menaquinone is MK-9(H₈). The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylinositol, three unidentified phospholipids, two unidentified phosphoglycolipids and two unidentified glycolipids. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}.

Table 1. Characteristics that differentiate strain K413^T from closely related species

Strains: 1, K413^T; 2, *S. marinus* DSM 41968^T; 3, *S. glycovorans* YIM M 10366^T; 4, *S. haliclona* DSM 41970^T; 5, *S. xishensis* YIM M 10378^T. All strains were positive for the ability to grow with D-mannitol as the sole carbon source (1.0 %) and for growth at pH 8, at 25, 28 and 37 °C, and in the presence of 1, 2 and 3 % (w/v) NaCl. All strains were negative for the ability to grow with L-methionine as the sole nitrogen source (0.1 %), growth at pH 4, at 4 and 10 °C, and in 15 % (w/v) NaCl. All data were obtained in this study. +, Positive; –, negative.

Characteristic	1	2	3	4	5
Biochemical tests					
Arbutin hydrolysis	+	+	+	+	–
Allantoin hydrolysis	+	–	–	–	+
Aesculin hydrolysis	–	+	–	–	–
Nitrate reduction	–	–	+	–	+
Urea	+	–	–	–	+
pH tolerance					
5	+	–	–	+	–
9	+	+	+	+	–
10	+	–	–	–	–
Temperature for growth (°C)					
45	–	–	+	–	–
NaCl tolerance (% w/v)					
4	+	+	–	+	+
5	+	+	–	+	+
6	–	+	–	+	+
7	–	+	–	+	+
8	–	+	–	+	–
9	–	+	–	+	–
10	–	+	–	+	–
Degradation of:					
Tween 20 (1 %)	–	–	–	+	+
Tween 80 (1 %)	–	–	–	+	–
Use of sole carbon sources (1.0 %, w/v)					
Cellobiose	+	–	–	+	+
D-Sorbitol	–	–	–	+	–
D-Galactose	+	+	–	+	–
Dextrin	+	+	+	+	–
Inulin	+	+	–	+	+
L-Arabinose	+	+	–	–	–
L-Rhamnose	+	–	–	–	–
Lactose	+	–	–	–	–
Maltose	–	–	+	–	+
Starch	+	–	–	–	–
Sucrose	–	+	+	+	+
Use of sole nitrogen sources (0.1 %, w/v)					
Glycine	+	–	–	–	–
L-Arginine	–	–	+	+	–
L-Hydroxyproline	+	–	–	–	–
L-Phenylalanine	+	–	+	–	–
L-Proline	+	–	–	–	–
L-Serine	–	–	+	+	+
L-Valine	+	–	+	–	–

The type strain, K413^T (=KCTC 29096^T=DSM 42068^T) was isolated from a soil sample taken from Karpaz National Park, Magusa, Northern Cyprus. The G+C content of the genomic DNA of the type strain is 71.9 mol%.

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References

- Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Busti, E., Monciardini, P., Cavaletti, L., Bamonte, R., Lazzarini, A., Sosio, M. & Donadio, S. (2006). Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. *Microbiology* **152**, 675–683.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chater, K. F., Biró, S., Lee, K. J., Palmer, T. & Schrempf, H. (2010). The complex extracellular biology of *Streptomyces* *FEMS Microbiol Rev* **34**, 171–198.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2005). A simple fluorimetric method for the estimation of DNA-DNA relatedness between closely related microorganisms by thermal denaturation temperatures. *Extremophiles* **9**, 75–79.
- Goodfellow, M. & Fiedler, H. P. (2010). A guide to successful bioprospecting: informed by actinobacterial systematics. *Antonie van Leeuwenhoek* **98**, 119–142.
- Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jones, K. L. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* **57**, 141–145.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.

- Kim, B.-Y., Zucchi, T. D., Fiedler, H.-P. & Goodfellow, M. (2012a). *Streptomyces cocklensis* sp. nov., a dioxamycin-producing actinomycete. *Int J Syst Evol Microbiol* **62**, 279–283.
- Kim, O.-S., Cho, Y.-J., Lee, K., Yoon, S.-H., Kim, M., Na, H., Park, S.-C., Jeon, Y. S., Lee, J. H. & other authors (2012b). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Labeda, D. P. (1992). DNA-DNA hybridization in the systematics of *Streptomyces*. *Gene* **115**, 249–253.
- Lam, K. S. (2007). New aspects of natural products in drug discovery. *Trends Microbiol* **15**, 279–289.
- Lechevalier, M. P. & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Nash, P. & Krent, M. M. (1991). Culture media. In *Manual of Clinical Microbiology*, 5th edn, pp. 1268–1270. Edited by A. Balows, W. J. Hausler, K. L. Herrmann, H. D. Isenberg & H. J. Shadomy. Washington, DC: American Society for Microbiology.
- Rateb, M. E., Houssen, W. E., Arnold, M., Abdelrahman, M.-H., Deng, H., Harrison, W. T. A., Okoro, C. K., Asenjo, J. A., Andrews, B. A. & other authors (2011). Chaxamycins A-D, bioactive ansamycins from a hyper-arid desert *Streptomyces* sp. *J Nat Prod* **74**, 1491–1499.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Santhanam, R., Rong, X., Huang, Y. & Goodfellow, M. (2013). *Streptomyces erringtonii* sp. nov. and *Streptomyces kaempferi* sp. nov., isolated from a hay meadow soil. *Antonie van Leeuwenhoek* **103**, 79–87.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Waksman, S. A. & Henrici, A. T. (1943). The nomenclature and classification of the actinomycetes. *J Bacteriol* **46**, 337–341.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.