Nocardiopsis fildesensis sp. nov., an actinomycete isolated from soil

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A filamentous actinomycete strain, designated GW9-2^T, was isolated from a soil sample collected from the Fildes Peninsula, King George Island, West Antarctica. The strain was identified using a polyphasic taxonomic approach. The strain grew slowly on most media tested, producing small amounts of aerial mycelia and no diffusible pigments on most media tested. The strain grew in the presence of 0–12 % (w/v) NaCl (optimum, 2–4 %), at pH 9.0–11.0 (optimum, pH 9.0) and 10–37 °C (optimum, 28 °C). The isolate contained *meso*-diaminopimelic acid, no diagnostic sugars and MK-9(H_4) as the predominant menaquinone. The major phospholipids were phosphatidylglycerol, phosphatidylcholine and phosphatidylmethylethanolamine. The major fatty acids were iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{18:1}\omega9c$, iso-$C_{15:0}$ and iso-$C_{17:0}$. DNA–DNA relatedness was 37.6 % with *Nocardiopsis lucentensis* DSM 44048^T, the nearest phylogenetic relative (97.93 % 16S rRNA gene sequence similarity). On the basis of the results of a polyphasic study, a novel species, *Nocardiopsis fildesensis* sp. nov., is proposed. The type strain is GW9-2^T (=CGMCC 4.7023^T=DSM 45699^T=NRRL B-24873^T).

The genus *Nocardiopsis* was initially described by Meyer (1976) and currently comprises 44 species with validly published names (http://www.bacterio.net/n/nocardiopsis.html). Members of the genus *Nocardiopsis* are ubiquitously distributed in the natural environment (Kroppenstedt & Evtushenko, 2002). The natural habitats of the described strains include soil (Kroppenstedt & Evtushenko, 2002; Hamedi *et al.*, 2010, 2011), marine environments (Sabry *et al.*, 2004; Chen *et al.*, 2009), plant tissue (Qin *et al.*, 2009), animal guts (Vasanthi & Hoti, 1992), clinical material (Bernatchez & Lebreux, 1991; Yassin *et al.*, 1997) and saline soils (Li *et al.*, 2003, 2004, 2006; Chen *et al.*, 2008). However, there has been only one species isolated from Antarctica (Abyzov *et al.*, 1983).

In the course of a screening program for novel species of the genus *Nocardiopsis*, one strain, designated GW9-2^T, was isolated from a soil sample collected from the west coast of the Fildes Peninsula near the Chinese Antarctic Great Wall Station, King George Island, West Antarctica. The diluted soil sample was inoculated onto Gause 1 agar (Du *et al.* 2008) [containing (per litre seawater): 20 g Starch, 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4.7H_2O$, 20 g agar (pH 7.4)] and incubated at 28 °C for 7 days. The isolated strain was routinely cultured on Gause 1 medium supplemented with 3 % (w/v) NaCl at 28 °C and stored at 4 °C. It was also stored as glycerol suspensions (20 %, v/v) at −160 °C in liquid nitrogen since the strain showed very reduced viability after storage at −20 °C or −80 °C.

The morphological characteristics of strain GW9-2^T were observed using light microscopy (model BH 2; Olympus) and scanning electron microscopy (model JSM-840; JEOL) after 14 days of growth on Gause 1 medium containing 3 % (w/v) NaCl. Cultural characteristics were determined after 4 weeks at 28 °C using the methods adopted by the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966). All media used were supplemented with 3 % (w/v) NaCl. Colours of substrate and aerial mycelia and diffusible pigments were determined by using colour chips from the Inter-Society Color Council–National Bureau of Standards (ISCC–NBS) colour charts (standard samples, no. 2106) (Kelly, 1964). The tolerance to different NaCl concentrations (0, 1, 2, 3, 4, 5, 7, 10, 12, 15 and 18 %, w/v) was tested on Gause 1 medium as the basal medium by incubating the cultures for 28 days at 28 °C. Growth at different temperatures (10, 16, 20, 25, 28 and 37 °C) and different pH (pH 5.0–11.0 at intervals of 1.0 pH unit) were tested on Gause 1 medium with 3 % (w/v) NaCl at 28 °C for 14–21 days. Media with different pH values were prepared using the buffer system described by Xu *et al.* (2005). Oxidase activity was determined from the oxidation of tetramethyl-*p*-phenylenediamine. Catalase activity was detected by the production of bubbles following addition of a drop of 3 % (v/v) H_2O_2. Hydrolysis of starch, gelatin and Tweens 20, 40 and 80 was determined as

The GenBank accession number of the 16S rRNA gene sequence of strain GW9-2^T is FJ853144.

Three supplementary tables and three supplementary figures are available with the online version of this paper.

described by Smibert & Krieg (1994). Carbon source utilization was determined using the GP2 MicroPlate system (Biolog) according to the manufacturer's instructions. Nitrogen source utilization was assessed according to the method of Williams *et al.* (1989). The detailed results are given in Table 1, Tables S1 and S2 (available in IJSEM Online), or in the species description.

Biomass for chemotaxonomic analyses was obtained by cultivation in shake flasks (with shaking at about 150 r.p.m.) using trypticase soy [3 % (w/v) NaCl, pH 9.0] broth at 28 °C for 1 week. Analyses of the amino acids and sugars of the cell walls were performed as described by Staneck & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1984). Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). The cellular fatty acid composition was determined according to the instructions of the Sherlock Microbial Identification System (MIDI Sherlock version 4.5, MIDI database TSBA40 4.10) (Kroppenstedt, 1985; Sasser, 1990). The genomic DNA for the determination of G+C content was prepared according to the method of Marmur (1961). The G+C content was determined by the thermal denaturation method of Marmur & Doty (1962). Levels of DNA–DNA relatedness were determined using the thermal denaturation and renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992).

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Cui *et al.* (2001). The 16S rRNA gene sequence of strain GW9-2^T was compared against a database of cultured species via BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzTaxon Database (server http://eztaxon-e.ezbiocloud.net/, Kim *et al.*, 2012). Neighbour-joining (Saitou & Nei, 1987) and

Table 1. Characteristics useful for differentiating between strain GW9-2^T and closely related species of the genus *Nocardiopsis*

Strains: 1, GW9-2^T; 2, *N. lucentensis* DSM 44048^T; 3, *N. alba* DSM 43377^T; 4, *N. halotolerans* DSM 44410^T; 5, *N. flavescens* SA6^T. The data are from this study, except for the cell-wall sugars, DNA G+C content, menaquinones and polar lipids of the reference strains. Experiments were performed under the same condition using the same methodology as used in this study. +, Positive; −, negative.

Characteristic	1	2	3	4	5
Growth temperature range (°C), optimum	16–37, 28	12–37, 28	12–37, 28	20–37, 30	23–40, 35
Growth pH range, optimum	5–11, 9	5–9, 7.3–7.5	5–10, 7.2	5–10, 7.2	5.5–11.0, 7.2–7.5
NaCl range for growth (%), optimum	0–12, 2–4	0–15, 5	0–10, 5	0–15, 10	0–10, 0–3
Gelatin liquefaction	−	+	+	+	+
Nitrate reduction	+	−	+	−	+
Milk coagulation, milk peptonization	−	+	+	+	−
H_2S production	−	−	−	+	−
Urease activity	−	+	+	−	−
Melanin production	−	−	−	+	−
Diagnostic sugars*	No characteristic sugars	No characteristic sugars	No characteristic sugars	Glucose and ribose	No characteristic sugars
Polar lipids*†	PG, PC, PME, PE	PC, PME, PG, DPG	PME, PC	PC, PI, PG, PME and four unknown	PC, PME, PG, DPG
Major menaquinones*	MK-9(H_4), MK-7, MK-10(H_4), MK-10(H_6)	MK-10(H_8), MK-10(H_4), MK-10(H_6), MK-10(H_{10})	MK-10(H_4), MK-10(H_6), MK-9(H_4)	MK-10, MK-10(H_2), MK-10(H_4), MK-10(H_6),	MK-10(H_2), MK-10(H_4)
Major fatty acids	iso-$C_{16:0}$ (19.3 %), anteiso-$C_{17:0}$ (12.7 %), $C_{18:1}\omega9c$ (9.3 %), iso-$C_{15:0}$ (8.3 %), iso-$C_{17:0}$ (8.3 %)	iso-$C_{16:0}$ (32 %), 10-methyl $C_{18:0}$ (15.4 %), anteiso-$C_{17:0}$ (14.5 %), C_{18} (13.1 %), iso-$C_{18:0}$ (9.4 %)	iso-$C_{16:0}$ (40 %), $C_{18:1}\omega9c$ (13 %), anteiso-$C_{17:0}$ (6 %), $C_{18:0}$ (7 %), iso-$C_{14:0}$ (5 %)	iso-$C_{16:0}$ (23 %), anteiso-$_{17:0}$ (18 %), anteiso-$_{15:0}$ (17 %), $C_{18:1}$ (9 %), $C_{17:1}$ (7 %)	anteiso-$C_{17:0}$ (28.1 %), iso-$C_{16:0}$ (18.7 %), $C_{18:0}$ (15.7 %), $C_{18:1}\omega9c$ (7.7 %)
DNA G+C content (mol%)*	76.8 %	71.0 %	70 %	68.0 %	68.6 %

*Data for the reference strains were taken from Yassin *et al.* (1993), Grund & Kroppenstedt (1990), Al-Zarban *et al.* (2002) and Fang *et al.* (2011).
†PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylmethylethanolamine; DPG, diphosphatidylglycerol.

maximum-parsimony (Fitch, 1971) trees were reconstructed using MEGA 4.0 (Tamura *et al.*, 2007), and a maximum-likelihood tree was generated using PHYLIP version 3.6 (Felsenstein 2002) after multiple alignment of data by CLUSTAL_X and CLUSTAL OMEGA (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) (Kimura, 1980) and clustering were determined using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

Strain GW9-2^T grew well on yeast extract–malt extract agar (ISP 2) and Czapek's agar. It showed moderate growth on inorganic salts–starch agar (ISP 4), glycerol–asparagine agar (ISP 5) and potato agar. No growth was observed on oatmeal agar (ISP 3) and nutrient agar (Table S1). Small amounts of white aerial mycelia formed slowly on ISP 2, ISP 4, ISP 5, potato agar and Czapek's agar, not on the other media tested. The substrate mycelia were yellow–white on ISP 2 and potato agar and white on ISP 4, ISP 5 and Czapek's agar. Soluble pigment was not produced on any of the media tested. The vegetative hyphae were long, well-developed and fragmented. Long spore chains were borne on the aerial hyphae. Spores (dimensions 0.6–0.8 × 0.8–1.0 mm) were short rod-shaped, rough and non-motile (Fig. S1). When tested on Gause 1 medium, growth of strain GW9-2^T was observed at temperatures between 16 and 37 °C, with an optimum growth temperature of 28 °C, and within the pH range pH 5.0–11.0, with an optimum of pH 9.0. The strain grew on Gause 1 medium with NaCl concentrations ranging from 0 to 12 % (w/v), with an optimum of 2–4 %. More physiological and biochemical characteristics of the strain are listed in Table 1 and in the species description.

Strain GW9-2^T contained *meso*-diaminopimelic acid as the diagnostic cell wall diamino acid and no diagnostic sugars. The major menaquinone was MK-9(H_4) (31 %) and other menaquinone components were MK-10(H_4) (14 %), MK-7 (13 %), MK-10(H_6) (9 %), MK-10(H_8) (8 %), MK-11 (4 %), MK-9(H_2) (3 %), MK-8(H_2) (2 %), MK-9 (2 %), MK-10 (2 %), MK-10(H_2) (2 %) and MK-8 (1 %). The phospholipid composition was phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylmethylethanolamine (PME) and other unidentified phospholipids (PLs) (Fig. S2). The major cellular fatty acids (>5 % of the total fatty acids) were iso-$C_{16:0}$ (19.3 %), anteiso-$C_{17:0}$ (12.7 %), $C_{18:1}\omega 9c$ (9.3 %), iso-$C_{15:0}$ (8.3 %) and iso-$C_{17:0}$ (8.3 %), which are typical major fatty acids of species of the genus *Nocardiopsis*. The G+C content of the genomic DNA of strain GW9-2^T was 76.8 mol%, which is in accordance with the values for the genus *Nocardiopsis* (70–76 mol%). The chemotaxonomic characteristics of strain GW9-2^T, such as amino acid and sugar of cell wall hydrolysates, major fatty acids and phospholipids were consistent with its assignment to the genus *Nocardiopsis*.

The almost-complete 16S rRNA gene sequence (1457 bp) of strain GW9-2^T was determined. The 16S rRNA gene sequences of the strain were compared against a database of cultured species via BLAST analysis and the EzTaxon-e server Database (Kim *et al.*, 2012) of type strains in order to retrieve the most similar sequences of recognized bacteria. Strain GW9-2^T showed high sequence similarities to *Nocardiopsis lucentensis* DSM 44048^T(97.93 %), followed by *Nocardiopsis flavescens* SA6^T (97.72 %), *Nocardiopsis alba* DSM 43377^T (97.65 %) and *Nocardiopsis halotolerans* DSM 44410^T (97.60 %). A neighbour-joining phylogenetic tree showed that the novel isolate occupied an independent branch in the phylogenetic tree (Fig. 1). This independent branch was also recovered with the other algorithms tested (Fig. S3A and B), which strongly indicated that the novel strain should not be assigned to any of the recognized species of the genus *Nocardiopsis*. DNA–DNA hybridizations were performed between the novel isolate and the type strains of *N. lucentensis* DSM 44048^T, *N. flavescens* SA6^T, *N. alba* DSM 43377^T and *N. halotolerans* DSM 44410^T to establish the precise taxonomic position of strain GW9-2^T. The DNA–DNA relatedness values were 37.6 ± 1.7 %, 34.9 ± 1.9 %, 33.8 ± 1.8 % and 33.3 ± 1.7 %, respectively, which were clearly below the 70 % threshold generally accepted for species delineation (Wayne *et al.*, 1987).

Strain GW9-2^T differed greatly from the other species in terms of some of its physiological and biochemical characteristics and some chemotaxonomic data (Table 1, Tables S1, S2 and S3). Strain GW9-2^T showed negative results in the tests for gelatin liquefaction, milk coagulation and milk peptonization, while *N. lucentensis* DSM 44048^T, *N. alba* DSM 43377^T and *N. halotolerans* DSM 44410^T showed positive results. The ability of strain GW9-2^T to reduce nitrate differed from *N. lucentensis* DSM 44048^T and *N. halotolerans* DSM 44410^T. Strain GW9-2^T show different result in the test for urea hydrolisis from *N. lucentensis* DSM 44048^T and *N. alba* DSM 43377^T. Differences in optimum pH and NaCl concentration for growth were also observed between strain GW9-2^T and its closest relatives. The major menaquinones of strain GW9-2^T were different from the components of its four closest relatives. Differences were also observed in fatty acid profiles (Table 1). Based on the phenotypic and genotypic results obtained in this study, it is concluded that strain GW9-2^T represents a novel species of the genus *Nocardiopsis*, for which the name *Nocardiopsis fildesensis* sp. nov. is proposed.

Description of *Nocardiopsis fildesensis* sp. nov.

Nocardiopsis fildesensis (fil.des.en′sis. N.L. fem. adj. *fildesensis* pertaining to the Fildes Peninsula, King George Island, West Antarctica, from where the type strain was isolated).

Gram-strain-positive and aerobic. The colour of the aerial mycelium is white on ISP 2, Czapek's agar, ISP 4, ISP 5 and potato agar and the substrate mycelium is white or yellowish white. No diffusible pigments are produced. The vegetative hyphae are long, well-developed and

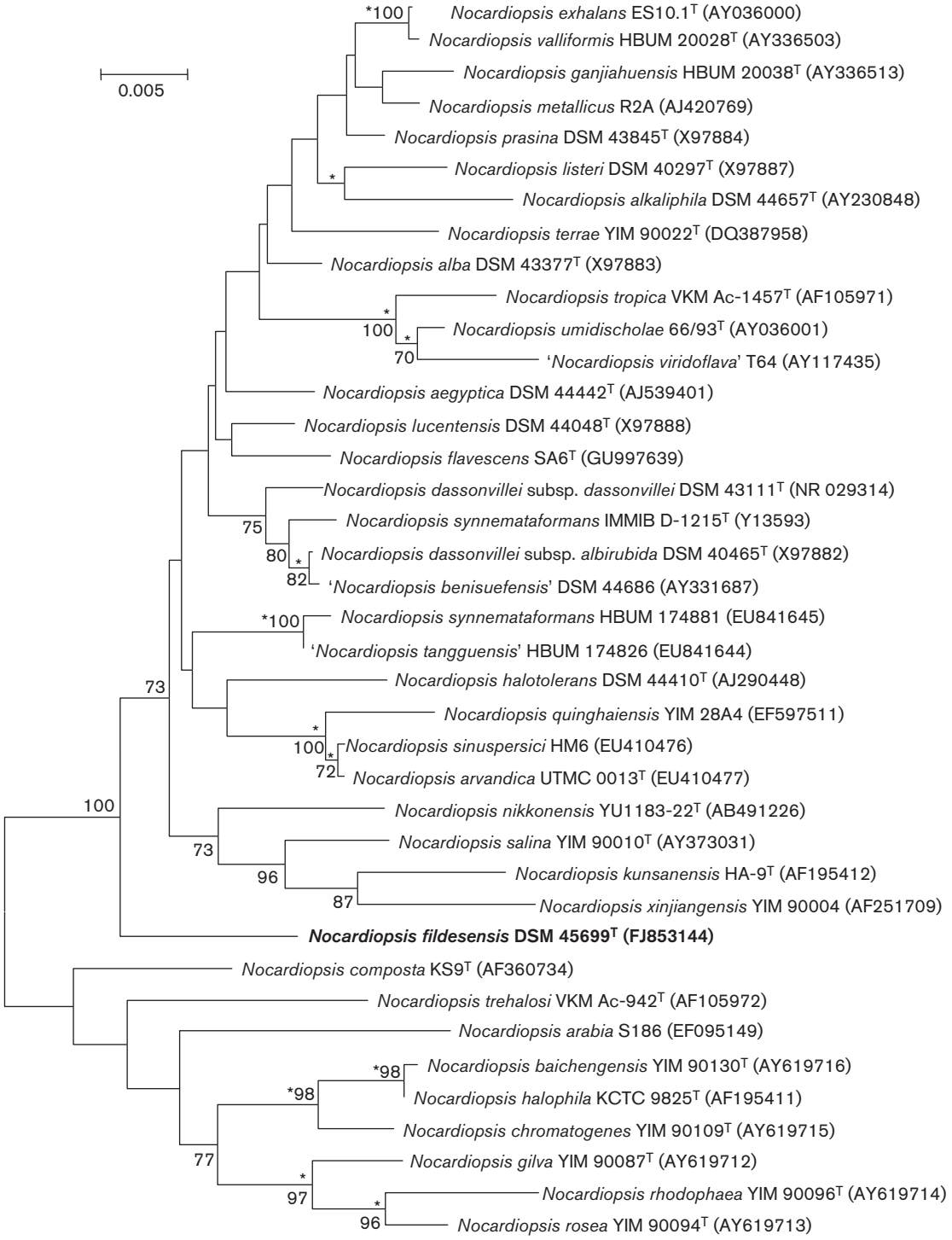

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain GW9-2^T within the genus *Nocardiopsis*. Asterisks indicate the clades that were conserved in the maximum-likelihood and maximum-parsimony trees. Bootstrap values are shown on nodes as percentages of 1000 replicates, when greater than 70 %. Bar, five substitutions per 1000 nt.

fragmented. Aerial mycelia differentiate into long spore chains. Spores (dimensions 0.6–0.8 × 0.8–1.0 mm) are rod-shaped, rough-surfaced and non-motile. Growth occurs at 16–37 °C (optimum, 28 °C), at pH 5.0–11.0 (optimum, pH 9.0) and in the presence of 0–12 % (w/v) NaCl (optimum, 2-4 %). Positive for nitrate reduction but negative for milk coagulation, peptonization, H_2S production and hydrolysis of gelatin and urea. The following substrates in the GP2 MicroPlate are utilized: D-fructose, D-galactose, D-mannose, sucrose, glucose, cellobiose, melibiose, D-mannitol, glycerol, trehalose, melezitose, glycogen, mannan, Tween 40, *N*-acetyl-β-D-mannosamine, cellobiose, L-fucose, D-gluconic acid, lactulose, maltotriose, 3-methyl-D-glucose, palatinose, sedoheptulosan, turanose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, L-lactic acid, *N*-acetyl-L-glutamic acid, D-alanine, L-glutamic acid and L-pyroglutamic acid. Cell walls contain *meso*-diaminopimelic acid and have no diagnostic sugars. Major polar lipids are phosphatidylglycerol, phosphatidylcholine and phosphatidylmethylethanolamine. The predominant respiratory quinone is MK-9(H_4). Major cellular fatty acids are iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{18:1}\omega9c$, iso-$C_{15:0}$ and iso-$C_{17:0}$.

The type strain, GW9-2^T (=CGMCC 4.7023^T=DSM 45699^T=NRRL B-24873^T), was isolated from a soil sample collected from the west coast of the Fildes Peninsula near the Chinese Antarctic Great Wall Station, King George Island, West Antarctica. The DNA G+C content of the genomic DNA of the type strain is 76.8 mol%.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31200009), the Chinese Polar Environment Comprehensive Investigation & Assessment Programmes (CHINARE2013-01-06-06), the Shandong Provincial Science and Technology Development Program (2009GG10002082) and by the Shandong Provincial Natural Science Foundation (ZR2010CM042). We also thank the Chinese Arctic and Antarctic Administration of the State Oceanic Administration for their support.

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