International Journal of Systematic and Evolutionary Microbiology (2014), 64, 4043–4048 DOI 10.1099/ijs.0.065870-0

# *Streptomyces burgazadensis* sp. nov., isolated from soil

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A novel actinobacterial strain, designated Z1R7$^T$, was isolated from a soil sample collected from Burgazada, in the Marmara Sea (Turkey), and the strain identity was determined using a polyphasic taxonomic approach. The organism had chemotaxonomic and morphological properties consistent with its classification in the genus *Streptomyces* and it formed a distinct phyletic line in the 16S rRNA gene tree, together with the type strains *Streptomyces specialis* GW41-1564$^T$ (95.76 %), *Streptomyces mayteni* YIM 60475$^T$ (95.64 %), *Streptomyces hainanensis* YIM 47672$^T$ (95.53 %), *Streptomyces hoynatensis* S1412$^T$ (95.29 %), *Streptomyces avicenniae* MCCC 1A01535$^T$ (94.74 %), *Streptomyces sedi* YIM 65188$^T$ (94.59 %) and *Streptomyces zhaozhouensis* NEAU-LZS-5$^T$ (94.68 %). Chomotaxonomic data revealed that strain Z1R7$^T$ possessed MK-9 ($H_8$) as the predominant menaquinone, LL-diaminopimelic acid as the diagnostic diamino acid, and galactose, glucose and ribose as whole cell sugars. Diphosphatidylglycerol, phoshphatidylethanolamine and phosphatidylinositol were the predominant polar lipids; iso-$C_{16:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$ were the major fatty acids, and the genomic DNA G+C content was 69.4 mol%. On the basis of these genotypic and phenotypic data, it is proposed that isolate Z1R7$^T$ (=KCTC 29434$^T$=DSM 42126$^T$) should be classified in the genus *Streptomyces* as *Streptomyces burgazadensis* sp. nov.

The genus *Streptomyces* represents a group of micro-organisms that are widely distributed in nature, encompassing nearly 630 species with validly published names (http://www.bacterio.net/index.html). The genus *Streptomyces* was first introduced by Waksman & Henrici (1943) to accommodate aerobic, spore-forming actinomycetes. Members of the genus *Streptomyces* are able to form an extensively branched substrate mycelium and are also able to produce aerial hyphae that typically differentiate into chains of spores. Members of the genus have LL-diaminopimelic acid in the cell-wall peptidoglycan, but no characteristic sugars (Lechevalier & Lechevalier, 1970) and possesses DNA rich in G+C (Williams *et al.*, 1983; Manfio *et al.*, 2003). Species of the genus *Streptomyces* are abundant in soil and are well known for their ability to produce biologically active secondary metabolites, including antibiotics, enzymes, enzyme inhibitors, antitumour agents and antifungal compounds (Bérdy, 2005; Goodfellow & Fiedler, 2010). In recent years, the search for novel pharmaceutical agents against drug-resistant microbes from common species of the genus *Streptomyces* has become a more difficult and costly task. Hence, more attention has been focused on isolating streptomycetes from unusual and unexplored environments, such as the oceans (Veyisoglu & Sahin, 2014) and desert soil (Santhanam *et al.*, 2013).

During an onging investigative project on the diversity of actinobacteria in Burgazada (40° 52′ 52″N 29° 4′ 8″E) in the Sea of Marmara, soil samples were collected from a *Pinus brutia* Ten. (Turkish pine) forest. Strain Z1R7$^T$ was isolated after incubation for 3 weeks at 28 °C on modified Bennett's agar (MBA; Jones, 1949) supplemented with filter-sterilized cycloheximide (50 μg $ml^{-1}$), nalidixic acid

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Streptomyces burgazadensis* Z1R7$^T$ is KF793919.

Three supplementary figures and three supplementary tables are available with the online Supplementary Material.

(50 μg $ml^{-1}$) and rifampicin (5 μg $ml^{-1}$). The strain was maintained on NZamine medium (medium no. 554; DSMZ) and yeast-malt extract agar [International *Streptomyces* Project medium 2 (ISP2); Shirling & Gottlieb, 1966] slopes at room temperature and stored as glycerol suspensions (20 %, v/v) at −20 °C.

The 16S rRNA gene was amplified using the universal primers 27F [5′-AGAGTTTGATC(AC)TGGCTCAG-3′] and 1492R [5′-ACGG(CT)TACCTTGTTACGACTT-3′] (Weisburg *et al.*, 1991) and the nucleotide sequence of strain Z1R7$^T$ was determined with an automated sequencer (model 377; Applied Biosystems). The 16S rRNA gene sequence of strain Z1R7$^T$ (1467 nt) was compared with corresponding sequences of representative reference strains of species of the genus *Streptomyces*. Identification of the closest phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012). A multiple alignment with sequences from closely related species was performed by using the program CLUSTAL W in MEGA5.0 (Tamura *et al.*, 2011). Phylogenetic trees were generated using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA5.0 (Tamura *et al.*, 2011). Evolutionary distances were calculated using the model of Jukes & Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Biomass for chemical studies was prepared by growing the strain in NZamine broth (medium no. 554; DSMZ) under aerobic conditions for 10 days at 28 °C in flasks on a rotary shaker at 160 r.p.m. Cells were harvested by centrifugation, washed twice in distilled water, recentrifuged and freeze-dried. The isomers of diaminopimelic acid in the cell wall and whole-cell sugars were analysed as described by Lechevalier & Lechevalier (1970, 1980) and Staneck & Roberts (1974), respectively. Cellular fatty acids were extracted, methylated and separated by GC using an Agilent Technologies 6890N instrument, fitted with an autosampler and a 6783 injector, according to the standard protocol of the Sherlock Microbial identification (MIDI) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) and the fatty acid methyl ester peaks were quantified using the TSBA 5.0 database. Polar lipids were extracted and analysed by the method of Minnikin *et al.* (1984), as modified by Kroppenstedt & Goodfellow (2006). The isoprenoid quinones were extracted and purified using the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1982). The DNA G+C content of strain Z1R7$^T$ was determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

Isolate Z1R7$^T$, *Streptomyces specialis* DSM 41924$^T$, *Streptomyces hainanensis* DSM 41900$^T$, *Streptomyces avicenniae* DSM 41943$^T$, *Streptomyces sedi* DSM 41942$^T$, *Streptomyces mayteni* KCTC 19383$^T$ and *Streptomyces hoynatensis* S1412$^T$ were examined for a broad range of phenotypic properties, as described by Williams *et al.* (1983). In addition, cultural characteristics of the test strains were recorded after incubation at 28 °C for 14 days on the standard media used in the ISP (Shirling & Gottlieb, 1966), modified Bennett's agar (MBA; Jones, 1949), Czapek's agar (Waksman,1967), nutrient agar (Oxoid) and tryptic soy agar (TSA; Difco). National Bureau of Standards (NBS) Colour Name Charts (Kelly, 1964) were used for determining colour designation and names. Colony morphology and the micromorphological properties of strain Z1R7$^T$ were determined (JSM 6060; JEOL SEM) by examining gold-coated, dehydrated specimens of 60-day-old cultures taken from ISP 4 medium, supplemented with vitamin B complex. Growth at different temperatures (4, 10, 20, 28, 37, 45 and 50 °C), pH 4.0–12.0 (at intervals of 1.0 pH unit), and in the presence of 0–10 % NaCl (w/v) was determined on yeast extract-malt extract (ISP 2). $KH_2PO_4$/HCl, $KH_2PO_4$/$K_2HPO_4$ and $K_2HPO_4$/NaOH buffer systems were used to maintain the pH of the media, while a NaCl buffer system was used for determining NaCl tolerance. The antimicrobial activity of strain Z1R7$^T$ in inhibiing the growth of nine micro-organisms, such as Gram-positive and Gram-negative bacteria, as well as fungi, was observed using the overlay technique described by Williams *et al.* (1983). Spot-inoculated colonies on modified Bennett's agar plates were inverted over 2 ml chloroform for 40 min. Killed colonies were overlaid with 5–7 ml sloppy modified Bennett's broth inoculated with the test organisms. Zones of inhibition were scored as being positive results after 24 h at 37 °C.

Comparison of the almost complete 16S rRNA gene sequence of isolate Z1R7$^T$ (1467 nt) with the corresponding sequences available on the EzTaxon server (Kim *et al.*, 2012), showed the highest 16S rRNA gene sequence similarity of 95.76 % with *S. specialis* DSM 41924$^T$, corresponding to 62 nt differences at 1462 sites. A neighbour-joining tree based on 16S rRNA gene sequences, which shows the position of strain Z1R7$^T$ and its closest phylogenetic relatives, is presented in Fig. 1. Strain Z1R7$^T$ formed an independent clade and a close subclade showed a corresponding branch composed of the type strains of *Streptomyces specialis* GW41-1564$^T$ (95.76 %), *Streptomyces mayteni* YIM 60475$^T$ (95.64 %), *Streptomyces hainanensis* YIM 47672$^T$ (95.53 %), *Streptomyces hoynatensis* S1412$^T$ (95.29 %), *Streptomyces avicenniae* MCCC 1A01535$^T$ (94.74 %), *Streptomyces zhaozhouensis* NEAU-LZS-5$^T$ (94.68 %) and *Streptomyces sedi* YIM 65188$^T$ (94.59 %); this was supported by maximum-likelihood and maximum-parsimony analysis with a high bootstrap value (>81 %) (Figs. S1 and S2, available in the online Supplementary Material).

The organism contained LL-diaminopimelic acid, with glucose, galactose and ribose present in whole-organism hydrolysates and the predominant quinone compound was MK-9 ($H_8$) (82.5 %). Minor amounts of MK-9 ($H_6$) (4.3 %), MK-9 ($H_2$) (3.9 %), MK-9 ($H_4$) (0.6 %) and an unspecified component (4.8 %) were also detected. The fatty acid profile contained major amounts of iso-$C_{16:0}$ (31.7 %), anteiso-$C_{17:0}$ (23.1 %) and anteiso-$C_{15:0}$ (13.9 %) acids (Table S1). The polar lipids were diphosphatidylglycerol, phoshphatidylethanolamine, phosphatidylinositol,

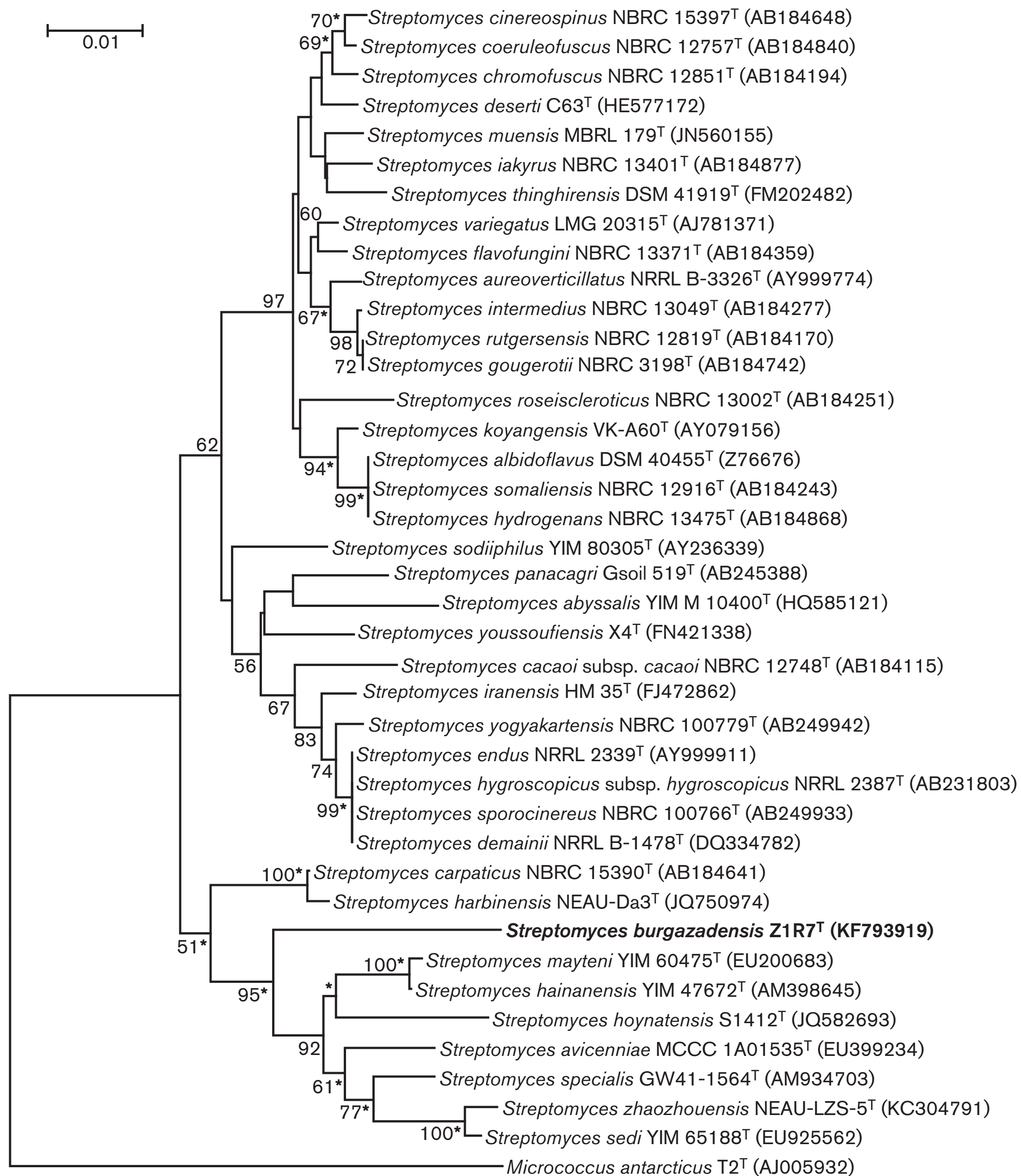


**Fig. 1.** Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing the position of strain S2704$^{T}$ amongst its phylogenetic neighbours. Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. *Micrococcus antarcticus* T2$^{T}$ (AJ005932) was used as an outgroup. Numbers at the nodes indicate the levels of bootstrap support (%); only values $\geqslant$50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per site.

a glycophospholipid and three glycolipids (i.e. phospholipid pattern type 2 *sensu* of Lechevalier *et al.*, 1977) (Fig. S3). The G+C content of the DNA was 69.4 mol%. All of these proporties are in line with the classification of the isolate in the genus *Streptomyces*.

The morphological features of isolate Z1R7$^{T}$ were consistent with its classification in the genus *Streptomyces* (Williams *et al.*, 1983; Kämpfer, 2012). After 60 days of incubation on ISP 4 medium supplemented with vitamin B complex, strain Z1R7$^{T}$ formed very weak white aerial mycelium, which differentiated into spiral chains of smooth-surfaced spores (Fig. 2). Aerial mycelia were not observed when it was grown on the other ISP media tested (ISP 2–7), or on Czapek's, modified Bennett's, nutrient or trypticase

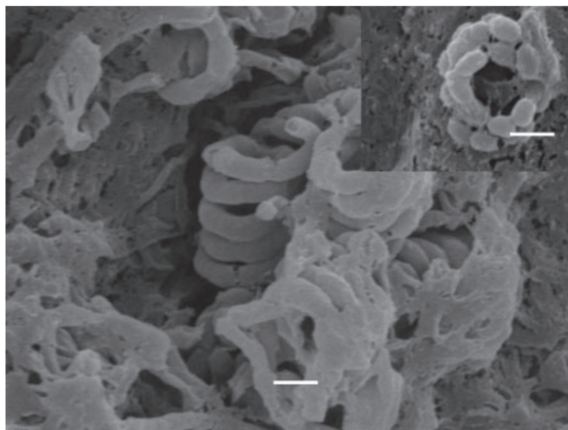

**Fig. 2.** Scanning electron micrograph of strain Z1R7$^T$ grown on inorganic salts-starch medium (ISP medium no. 4) supplemented with vitamin B complex, at 28 °C for 60 days. Bars, 1 μm.

soy media. Strain Z1R7$^T$ showed good growth on modified Bennett's, ISP 2, 4 and 5 and Czapek's agars, and moderate growth on tryptic soy, nutrient and ISP 7 media, but no growth was observed on ISP 3 and ISP 6. The colour of the substrate mycelium was yellowish to orange–yellow on ISP 2, 4, 5 and 7, nutrient, trypticase soy, modified Bennett's and Czapek's agars. Light orange and vivid yellowish-pink diffusible pigments were produced on ISP 4 and ISP 7, respectively. Melanoid pigments were not produced on ISP 6 or ISP 7 medium. The physiological and biochemical properties of Z1R7$^T$ are given in Tables 1, S2 and S3, and in the species description.

It is evident that strain Z1R7$^T$ is clearly different from all other species of the genus *Streptomyces*. It shares less than 96 % 16S rRNA gene sequence similarity with all species of the genus *Streptomyces* with validly published names and, on the basis of several phenotypic characteristics, it could be differentiated clearly from its phylogenetic neighbours *Streptomyces specialis* DSM 41924$^T$, *Streptomyces mayteni* KCTC 19383$^T$, *Streptomyces hainanensis* DSM 41900$^T$, *Streptomyces hoynatensis* S1412$^T$, *Streptomyces avicenniae* DSM 41943$^T$ and *Streptomyces sedi* DSM 41942$^T$ (Tables 1 and S2). Based on phylogenetic and phenotypic evidence, strain Z1R7$^T$ represents a novel species within the genus *Streptomyces*, for which the name *Stretomyces burgazadensis* sp. nov. is proposed.

## Description of *Streptomyces burgazadensis* sp. nov.

***Streptomyces burgazadensis*** (bur.ga.zad.en′sis. N.L. masc. adj. *burgazadensis* from the Burgazada, Istanbul, Turkey).

Aerobic, Gram-stain-positive, non-motile, non-acid-alcohol-fast actinomycete, which forms branched substrate hyphae and aerial mycelium that differentiates into spiral chains with smooth-surfaced spores (0.4–0.6 × 0.6–1.0 μm).

**Table 1.** Phenotypic properties of strain Z1R7$^T$ and closely related type species

Strains: 1, Z1R7$^T$; 2, *Streptomyces specialis* DSM 41924$^T$; 3, *Streptomyces mayteni* KCTC 19383$^T$; 4, *Streptomyces hainanensis* DSM 41900$^T$; 5, *Streptomyces hoynatensis* S1412$^T$; 6, *Streptomyces avicenniae* DSM 41943$^T$; 7, *Streptomyces sedi* DSM 41942$^T$. Strains were positive for the degradation of Tween 40 (1.0 %) and Tween 80 (1.0 %), and growth at pH 7, pH 8 and pH 9, as well as at a temperature of 28 °C and without NaCl. But they were negative for the degradation of xanthine (0.4 %) and xylan (0.4 %), for the hydrolysis of allantoin, and the ability to utilize inulin, dextran, L-glutamic acid and sorbose as sole carbon sources (1.0 %), or L-threonine as the sole nitrogen source (0.1 %). Strains did not grow at pH 4 or 12, or at 4, 10 or 50 °C. All data were obtained in this study. +, Positive; −, negative

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Biochemical tests | | | | | | | |
| Arbutin hydrolysis | + | − | − | − | − | − | − |
| Nitrate reduction | + | − | − | − | − | − | + |
| Urea | − | − | − | − | − | − | + |
| pH Tolerence | | | | | | | |
| 11 | + | − | − | − | − | − | − |
| Temperature for growth (°C) | | | | | | | |
| 20 | − | − | + | + | − | + | − |
| 37 | + | + | − | − | + | + | + |
| 45 | − | − | − | − | + | − | − |
| Carbon source utilization (1.0 %, w/v) | | | | | | | |
| Adonitol | + | + | + | − | − | + | + |
| *myo*-Inositol | - | + | - | - | - | - | - |
| D-Arabinose | + | + | − | + | + | − | − |
| L-Arabinose | + | − | − | − | + | + | + |
| Cellobiose | + | − | + | + | + | + | + |
| D-Fructose | + | − | + | − | + | + | + |
| D-Sorbitol | − | + | − | − | − | + | − |
| D-Galactose | + | − | + | − | + | + | + |
| Melezitose | + | − | − | − | + | + | + |
| L-Rhamnose | + | − | − | − | + | + | − |
| Sucrose | − | + | + | − | + | + | + |
| Xylitol | + | + | − | − | − | − | − |
| Xylose | + | − | − | − | + | + | − |
| Nitrogen source utilization (0.1 %, w/v) | | | | | | | |
| α-Isoleucine | + | − | − | − | + | + | − |
| Glycine | + | − | − | − | + | − | − |
| L-Alanine | + | − | − | − | + | + | + |
| L-Arginine | + | + | + | + | + | + | − |
| L-Cysteine | + | − | − | − | − | − | + |
| L-Histidine | + | − | + | − | − | − | + |
| Hydroxy-L-proline | + | − | − | − | + | + | − |
| L-Methionine | + | − | + | − | + | + | − |
| L-Phenylalanine | + | − | − | − | + | − | − |
| L-Proline | + | − | + | − | − | − | + |
| L-Serine | + | − | − | − | + | + | + |
| L-Valine | + | − | − | − | + | + | − |
| L-Tyrosine | + | + | − | − | + | + | + |

Growth occurs at pH 7.0–11 and at 28–37 °C, but not at pH 4, 5, 6 or 12 or at temperatures of 4, 10, 20, 45 or 50 °C. Optimum growth occurs on NZamine medium (medium 554; DSMZ) at 28 °C and pH 7.2. NaCl is not required for optimal growth. Arbutin is hydrolysed, but allantoin and urea are not hydrolysed. Nitrate reduction is positive. Starch and Tweens 40 and 80 are degraded, but adenine, casein, guanine, hypoxanthine, xanthine and xylan are not. Utilizes adonitol, D-arabinose, L-arabinose, cellobiose, D-fructose, D-galactose, D-mannitol, dextrin, melezitose, L-rhamnose, lactose, maltose, xylose and xylitol as sole carbon sources, but not *myo*-inositol, D-sorbitol, inulin, dextran, L-glutamic acid, sorbose, sucrose and succinic acid. Utilizes α-isoleucine, glycine, L-alanine, L-arginine, L-cysteine, L-histidine, hydroxy-L-proline, L-methionine, L-phenylalanine, L-proline, L-serine, L-valine and L-tyrosine as sole nitrogen sources. Antimicrobial activity is shown against *Aspergillus niger* (clinic isolate), *Candida utilis* NRRL Y-900, *Escherichia coli* MC4100, *Enterobacter aerogenes* NRRL B-3567, *Pseudomonas aeruginosa* NRRL B-2679, *Bacillus subtilis* NRRL B-209 and *Staphylococcus aureus* ATCC 33862. The predominant menaquinone of the type strain is MK-9 ($H_8$) with minor amounts of MK-9 ($H_6$), MK-9 ($H_2$) and MK-10 ($H_4$). The polar lipid profile contains diphosphatidylglycerol, phoshphatidylethanolamine, phosphatidylinositol, glycophospholipid and three glycolipids. The major cellular fatty acids are iso-$C_{16:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$.

The type strain, Z1R7$^T$ (=KCTC 29434$^T$=DSM 42126$^T$) was isolated from a soil sample collected from Burgazada, Istanbul, Turkey. The G+C content of the genomic DNA of the type strain is 69.4 mol%.

## Acknowledgements

This research was supported by Ondokuz Mayis University (OMU), project no. PYO. FEN. 1904.13.004.

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