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TAXONOMIC DESCRIPTION
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Streptomyces coryli sp. nov., isolated from hazelnut orchard soil

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Abstract

A novel actinobacteria, isolate A7024^T, was isolated from commercial hazelnut orchard soil sample which was collected at Duzce, West Black Sea region, Turkey. A polyphasic taxonomic study was carried out to determine the status of this isolate. The phylogenetic tree reconstructed using the neighbour-joining algorithm based on 16S rRNA gene sequences indicated that isolate A7024^T was positioned within the members of the genus *Streptomyces* with the highest sequence similarity (97.7%) to *Streptomyces cadmiisoli* ZFG47^T. The organism formed an extensively branched substrate and aerial hyphae which generated irregular rod-shaped spores with smooth-surfaces. The cell wall of strain A7024^T contained LL-diaminopimelic. Glucose, mannose and ribose were detected as whole-cell sugars. Its polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, three unidentified phospholipids and three unidentified glycolipids. Major menaquinones were MK-9(H_6) and MK-9(H_4). The major cellular fatty acids were iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. Strain A7024^T had a genome size of 9.0 Mb with a genome G+C content of 71.5 mol%. The low level of 16S rRNA gene similarity, 19.3 ± 2.3% digital DNA–DNAhybridization and 76.94% average nucleotide identity values, as well as some different phenotypic characteristics allowed the strain to be distinguished from the closely related type strains. Therefore, it is concluded that strain A7024^T represents a novel species of the genus of *Streptomyces*, for which the name *Streptomyces coryli* sp. nov. is proposed. The type strain is A7024^T (=DSM 42066^T=KCTC 29102^T=NRRL B-24888^T).

Streptomyces is the largest genus of the phylum *Actinobacteria* [1], and this genus encompasses aerobic Gram-stain-positive bacteria that form an extensively branched substrate mycelium. At the time of writing, the genus consists of 656 validly published and named species [2] (https://lpsn.dsmz.de/genus/streptomyces). The members of the genus characterized by: cell wall Type I [3]; DNA with high G+C content; and diphosphatidylglycerol, phospatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids [4–6]. Members of the genus *Streptomyces* are widely described among *Actinobacteria* as antibiotic producers [7]. Therefore, many studies have aimed to reveal the isolation, identification and characterization of members of the genus *Streptomyces* [8–14].

This study aimed to establish the taxonomic position of actinobacteria from hazelnut orchard soil samples. One of the isolates, A7024^T, was found to be a novel species of the genus *Streptomyces*, named here *Streptomyces coryli* sp. nov.

ISOLATION AND MAINTENANCE

A soil sample was collected from a commercial hazelnut orchard, (40° 49′ 00.80″ N 30° 57′ 36.64″ E) Duzce, Turkey. The sample was air-dried at room temperature for 14 days and suspended with ¼ strength Ringer's solution (Oxoid) to prepare 10^{-1} dilution, and then the dilution was shaken for an hour. After shaking, it was heated at 55 °C for 20 min and serial dilutions were performed until 10^{-4} was prepared. The diluted soil suspension (200 µl) was inoculated on Stevenson's medium no. 3 [15] supplemented with cycloheximide (50 µg ml^{-1}), nalidixic acid (10 µg ml^{-1}), nystatin (50 µg ml^{-1}) and novobiocin (10 µg ml^{-1}), and incubated at 28 °C for 21 days. Strain A7024^T, an actinobacterial colony, was selected and

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Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; ISP, International *Streptomyces* Project; MLSA, multilocus sequence analysis.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A7024^T is JN989307. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAAKZV000000000. The version described in this paper is version JAAKZV010000000.
Three supplementary figures and one supplementary table are available with the online version of this article.

purified on modified Bennett's agar [16], and then held into glycerol suspensions (20%, v/v) at −80 °C for long-term storage.

MOLECULAR CHARACTERIZATION

Genomic DNA was extracted from cells grown on International *Streptomyces* Project (ISP) 2 broth via shaking incubator at 28 °C for 7 days. The 16S rRNA gene amplification was carried out as described by Chun and Goodfellow [17]. The almost-complete 16S rRNA gene sequence (1469 bp) of strain A7024^T was determined using an ABI PRISM 3730 XL automatic sequencer. To reveal the phylogenetic relationship, the obtained sequence was compared with sequences of other type strains available in the EzBioCloud server [18] (www.ezbiocloud.net/). Multiple alignments with sequences from closely related strains were performed by using CLUSTAL W in MEGA version 7.0 software [19]. The five housekeeping gene (*atpD*, *gyrB*, *recA*, *rpoB* and *trpB*) sequences were directly downloaded from GenBank or retrieved from draft/complete genome sequences to determine multilocus sequence analysis (MLSA) evolutionary distances. Phylogenetic trees were reconstructed with three different algorithms (neighbour-joining [20], maximum-parsimony [21] and maximum-likelihood [22]) in MEGA 7.0. For maximum-parsimony analysis, subtree-pruning–regrafting was used, and for maximum-likelihood analysis, the nearest-neighbour-interchange heuristic algorithm was used for tree searching. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The 16S rRNA gene sequence of *Kitasatospora nipponensis* HKI 0315^T (AY442263) was used as an outgroup. Evolutionary distance matrices were generated using the model of Jukes and Cantor [23]. Topologies of the resultant trees were evaluated by bootstrap analysis [24] based on 1000 resamplings.

The genome of strain A7024^T was sequenced using Illumina HiSeq 2500 appartus with 250 bp paired-end reads at MicrobesNG (Birmingham, UK). SPAdes was used to assemble the raw sequence reads on the PATRIC web server [25] (https://patricbrc.org/). Contigs with a coverage value less than 5× and a length shorter than 500 bp were discarded. The draft genome sequence of strain A7024^T was submitted to the NCBI under the accession number JAAKZV000000000. The genome sequence was annotated on the Rapid Annotations Using Subsystems Technology (RAST) server [26]. Predictions of biosynthetic gene clusters were performed on antiSMASH version 5.1.0 [27]. Digital DNA–DNA hybridization (dDDH) and the average nucleotide identity (ANI) values were determined using Formula 2 of the Genome-to-Genome Distance Calculator 2.1 (http://ggdc.dsmz.de/ggdc.php) [28] and ANI Calculator [29], respectively.

Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain A7024^T was a member of the genus *Streptomyces*. The closest-related type species was found to be *Streptomyces cadmiisoli* ZFG47^T (97.72%) with a similarity value which can be considered too low. The neighbour-joining trees based on the 16S rRNA gene sequences revealed that A7024^T could be differentiated from *S. cadmiisoli* ZFG47^T and clustered together with *Streptomyces boninensis* K11-0400^T (97.65%) and *Streptomyces polyrhachis* NEAU-ycm1^T (97.10%) (Figs 1 S1 and S2, available in the online version of this article). All of these similarity values shared with *S. cadmiisoli* ZFG47^T, *S. boninensis* K11-0400^T and *S. polyrhachis* NEAU-ycm1^T are below the threshold of 98.65% for delineation of novel species [30, 31]. Similarly, strain A7024^T was differentiated from closely related type strains in the MLSA phylogenetic tree (Fig. 2).

The whole genome sequence of the strain A7024^T presents a genome size of 9031846 bp, comprising 613 contigs, with 8566 genes and 65 RNA. The N50 value is 28409 bp and the L50 value is 92 contigs. The DNA G+C content of strain A7024^T was found to be 71.5 mol%. The DNA–DNA relatedness, OrthoANI and MLSA genetic distance values between strain A7024^T and *S. cadmiisoli* ZFG47^T are 19.3 ± 2.3%, 76.94% and 0.137, respectively. All of these results are significantly below 70% [32] and 95~96% [33] recommended cut-off points (for dDDH and ANI values, respectively) and above the threshold of 0.007 MLSA evolutionary distance proposed by Rong and Huang [34] for the delineation of bacterial species.

The genome of strain A7024^T was rich in NRPS (eight clusters) and terpene (four clusters) types and contained 26 potential biosynthetic gene clusters. Among the biosynthetic gene clusters of strain 16K104^T, one cluster showed 84% similarity to genes coding for feglymycin, which consistently inhibits HIV replication in the lower μM range [35]. Moreover, one NRPS biosynthetic gene cluster of the strain contained genes sharing 10% similarity to genes encoding conglobatin, which shows antitumour activity [36].

CHEMOTAXONOMY

Biomass for chemotaxonomic analysis was prepared by growing strain A7024^T in ISP2 broth [37] at 160 r.p.m., 28 °C for 14 days. After cells were harvested by centrifugation and washed twice in distilled water, they were re-centrifuged and freeze-dried. The isomers of diaminopimelic acid [38] and whole-cell sugar composition [39] were analysed by TLC, using standard chemotaxonomic techniques. Polar lipid and respiratory quinones analyses were carried out by the Identification Service of the Leibniz Institute DSMZ (Braunschweig, Germany) following the procedures described by Tindall [40, 41] and Minnikin *et al.* [42], respectively. Fatty acids from strain A7024^T were extracted, methylated and analysed by gas chromatography using an Agilent Technologies 6890 N system, fitted with G2614A autosampler and 6783 injector, according to the standard protocol of the Microbial Identification System (MIDI) and Sherlock software version 6.1 with the TSBA5 database [43, 44].

Strain A7024^T contained LL-diaminopimelic acid as the cell-wall diamino acid, and was hence type-I [3]. Whole-cell

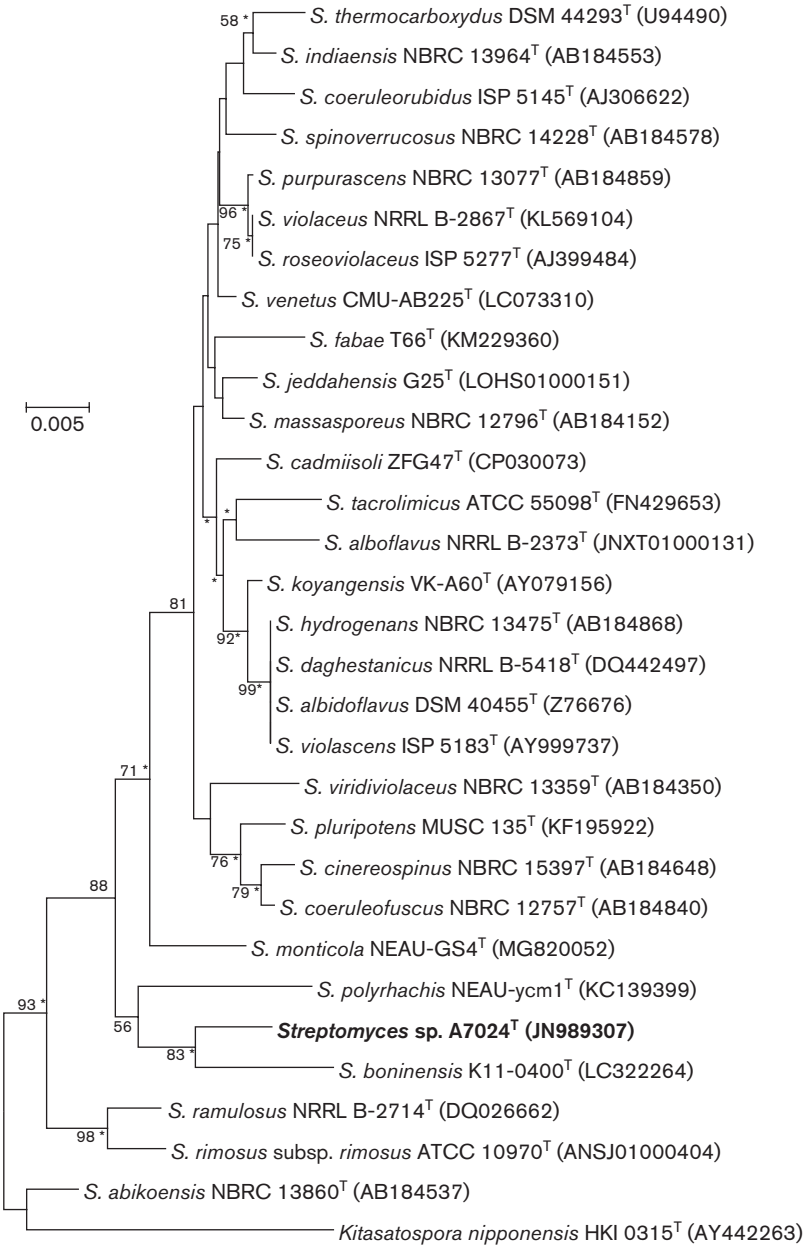

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain A7024^T and type strains of the genus *Streptomyces*. *Kitasatospora nipponensis* HKI 0315^T was used as an outgroup. All positions containing gaps and missing data were eliminated. There were a total of 1365 positions in the final dataset. Bootstrap percentages based on 1000 replicates are shown; values ≥50% are shown. Bar, 0.005 substitutions per nucleotide position. Asterisks (*) indicate clades that were conserved in the neighbour-joining, maximum-likelihood and maximum-parsimony trees. Accession numbers are indicated in parentheses.

hydrolysates contained glucose, ribose and mannose. The polar lipids of strain A7024^T consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, three unidentified phospholipids and three unidentified glycolipids (Fig. S3). The menaquinones of strain A7024^T were MK-9(H_6) (54%) and MK-9(H_4) (29%) with minor amounts of MK-9(H_8) (6%), MK-9(H_2) (3%) and MK-9 (2%). The major cellular fatty acids (>10%) of strain A7024^T were iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. Detailed fatty acid profiles are shown in Table 1.

CULTURAL, MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES

Cultural characteristics of strain A7024^T were tested on ISP2–7 media [37], Czapek's solution agar [45], modified

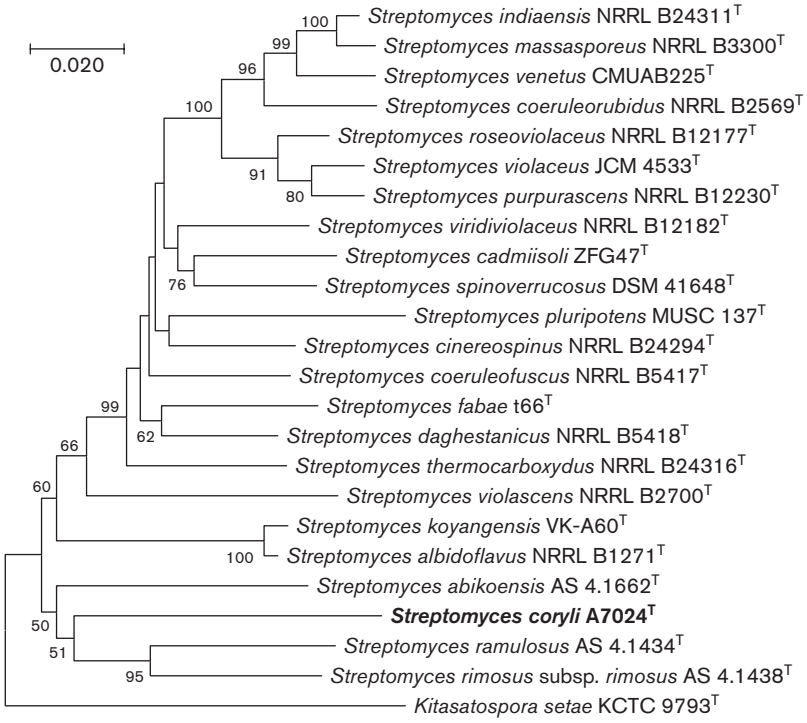

Fig. 2. *atpD-gyrB-recA-rpoB-trpB* phylogenetic tree showing the position of strain A7024^T within the genus *Streptomyces*. *Kitasatospora setae* KCTC 97935^T was used as outgroup. The tree was reconstructed using the neighbour-joining method based on 2097 bp sequence. The analysis involved 24 nucleotide sequences. All positions containing gaps and missing data were eliminated. Numbers at the nodes indicate percentages of bootstrap support; only values over 50% are shown. Bar, 0.02 substitutions per nucleotide position.

Bennett's agar [16], nutrient agar [46] and tryptic soy agar (TSA; Difco) after incubation for 14 days at 28 °C. Morphological properties of isolate A7024^T were observed by light microscopy and a JEOL JSM-6060 scanning electron microscope to examine gold-coated dehydrated specimens. The samples were prepared from the cultures grown for 21 day on ISP3 medium [37] at 28 °C. Growth was determined at various temperatures (4, 10, 20, 28, 37, 40, 45, 50 and 55 °C), different pH (pH 4.0–12.0) and concentrations of NaCl (up to 10%, w/v, at intervals of 1.0% unit) on ISP2 agar after incubation for 14 days. KH_2PO_4/HCl, KH_2PO_4/K_2HPO_4 and K_2HPO_4/NaOH buffer systems were used to maintain the pH values of the broth culture. Degradation tests were evaluated using the methods described by Nash and Krent [47] and Williams *et al*. [48]. Utilization of carbohydrates as sole carbon sources at a final concentration of 1% (w/v) was performed according to the methods proposed by Shirling and Gottlieb [37]. Nitrogen source utilization was examined according to Williams *et al*. [48] with a final concentration of 0.1% (w/v) for each nitrogen source. Additionally, antimicrobial activity of strain A7024^T to inhibit the growth of 15 micro-organisms, including Gram-positive and Gram-negative bacteria as well as fungi was observed using an overlay technique according to the procedures described by Williams *et al*. [48]. 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 positive results after 48 h incubation at 37 °C.

The morphological properties of strain A7024^T were indicated that the strain was typical for the genus *Streptomyces*. Strain A7024^T formed an extensively branched substrate and aerial mycelia. The colours of the substrate mycelia were observed to be brown. The aerial mycelium consisted of hyphae which fragmented into irregular rod-shaped spores with smooth surfaces (0.4–0.6×0.8–1.1 µm) (Fig. 3). While growth was good in other media, moderate growth was observed in TSA, ISP3, 4 and 6 media. Diffusible and melanoid pigments were not detected (Table S1). Strain A7024^T was found to grow between at pH 5.0–10.0, 10–37 °C and with 0–5% (w/v) NaCl. Additional phenotypic properties are given in the species description and Table 2. Strain A7024^T was found to exhibit antimicrobial activity against *Pseudomonas aeruginosa* NRRL B-2679, *Bacillus cereus* NRRL B-3711, *Bacillus licheniformis* NRRL B-1001, *Listeria monocytogenes* ATCC 19117 and *Micrococcus luteus* NRRL B-1018.

The chemotaxonomic characteristics, such as major menaquinones, whole-cell sugars and polar lipids, of A7024^T are generally compatible with the genus *Streptomyces*. However,

Table 1. Fatty acid profiles of strain A7024^T and themost closely related type strain.

Strains: 1, A7024^T; 2, *Streptomyces cadmiisoli* ZFG47^T. Cells were grown in ISP2 broth on a rotary shaker at 160 r.p.m. for 14 days at 28 °C. –, Not detected.

Fatty acids	1	2‡
Saturated		
$C_{14:0}$	–	1.5
$C_{16:0}$	4.3	15.4
$C_{17:00}$	–	2.7
$C_{17:1}$ ω8*c*	–	1.4
Branched		
cyclo-$C_{17:0}$	–	0.5
iso-$C_{14:0}$	6.5	6.1
iso-$C_{15:0}$	4.3	5.5
iso-$C_{16:0}$	35.2	30.4
iso-$C_{17:0}$	1.0	2.6
iso-$C_{18:0}$	–	1.1
iso-$C_{16:1}$ H	3.7	2.2
anteiso-$C_{15:0}$	30.4	10.9
anteiso-$C_{17:0}$	10.0	5.5
anteiso-$C_{17:1}$C	1.5	–
anteiso-C17:1 ω9*c*	–	1.4
Summed feature 3*	–	4.9
Summed feature 9†	–	1.8

*Summed feature 3 comprises $C_{16:1}\omega$6c or $C_{16:1}\omega$7c.
†Summed feature 9 comprises iso-$C_{17:1}\omega$9c and 10-methyl $C_{16:0}$.
‡Data taken from Li *et al.* [49].

16S rRNA gene sequence identities of strain A7024^T with all current validly named *Streptomyces* strains are well below 98.65% and this indicates that the strain should be novel species [30, 31]. Low DNA–DNA relatedness and OrthoANI values and too high MLSA evolutionary distances values as well as the differential characteristics given in Table 2 support this novelty. Thus, it is concluded that strain A7024^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces coryli* sp. nov. is proposed.

DESCRIPTION OF *STREPTOMYCES CORYLI* SP. NOV.

Streptomyces coryli (co.ry'li. L. gen. n. *coryli* of the hazelnut).

Aerobic, Gram-stain-positive, catalase-positive, non-motile actinobacterium that forms extensively branched substrate and aerial mycelia. Vegetative mycelium appears brown. Aerial mycelium forms irregular rod-shaped spores

Fig. 3. Scanning electron microscopy image of strain A7024^T grown on ISP3 agar at 28 °C for 21 days. Bar, 1 µm.

Table 2. Differential characteristics of strain A7024T and type strain of closely related species in the genus *Streptomyces*

Strains: 1, A7024^T; 2, *Streptomyces cadmiisoli* ZFG47^T.

Characteristics	1	2*
Isolation source	Hazelnut soil	Cadmium-rich soil
NaCI tolerance (%, w/v)	0–5	0–12
Temperature range for growth (°C)	10–37	15–40
Degradation of (1.0 %, w/v):		
Tween 80	–	+
Carbon source utilization (1.0%, w/v):		
D-Mannitol	+	–
Nitrogen source utilization (0.1%, w/v):		
L-Threonine	–	+
Major menaquinones (>10%)	MK-9 (H_6), MK-9(H_4)	MK-9 (H_4), MK-9(H_8)
Polar lipids†	DPG, PE, PI	DPG, PG, PE, PIM
Major fatty acids (>10%)	iso-$C_{16:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{15:0}$, $C_{16:0}$
Whole-cell sugars‡	Glu, Man, Rib	Gal, Glu, Man, Xyl

*Data taken from Li *et al.* [49].
†DPG, diphosphatidylglycerol; PE; phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PG, phosphatidylglycerol.
‡Gal, galactose; Glu, glucose; Man, mannose; Rib, ribose; Xyl, xylose.

(0.4–0.6×0.8–1.1 µm) with smooth surfaces. Good growth is observed on modified Bennett's, Czapek's, nutrient and ISP2, 5 and 7 media, and moderate growth is observed on TSA and ISP3, 4 and 6 media. Melanoid pigments are not produced on ISP6 or 7 agars. Growth occurs at 10–37 °C (optimum, 28 °C), at pH 5.0–10.0 (optimum, pH 7.0) and in the presence of 0–5% (w/v) NaCI. Allantoin and aesculin are hydrolysed, but arbutin is not. Negative in tests for production of H_2S and reduction of nitrate. Elastin, hypoxanthine, starch and Tween 20 are degraded, but not guanine or Tween 80. Adonitol, L-arabinose, cellobiose, dextrin, D-galactose, D-glucose, inulin, D-mannose, lactose, maltose, D-mannitol, L-rhamnose and sucrose are utilized as sole carbon and energy sources but not melezitose or D-sorbitol. α-Isoleucine, L-alanine, L-arginine, L-asparagine, L-cysteine, L-methionine, L-phenylalanine, L-proline, L-serine and L-valine are utilized as sole nitrogen sources, but not glycine, L-hydroxyproline, L-threonine or L-tyrosine. The polar lipid profile includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, three unidentified glycolipids and three unidentified phospholipids. The fatty acid profile consists mainly of iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The diagnostic diaminopimelic acid is LL-DAP. Whole-cell sugars contain glucose, mannose and ribose. Predominant menaquinones are MK-9 (H_6) and MK-9(H_4).

The type strain, A7024^T (=KCTC 29102^T=DSM 42066^T=NRRL B-24888^T), was isolated from soil collected from a commercial hazelnut orchard in Duzce, Turkey. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and the genome of strain A7024^T are JN989307 and JAAKZV000000000, respectively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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