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SHORT COMMUNICATION

Rathayibacter tanaceti sp. nov., a Novel Actinobacterium from *Tanacetum vulgare* Infested by Foliar Nematode *Aphelenchoides* sp.

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Abstract

Two novel yellow-pigmented, rod-shaped and non-motile coryneform actinobacteria, strains VKM Ac-2596^T and VKM Ac-2761, were isolated from a plant *Tanacetum vulgare* (Asteraceae) infested by foliar nematode *Aphelenchoides* sp. The strains exhibited the highest 16S rRNA gene sequence similarities to *Rathayibacter agropyri* CA4^T (99.71%), *Rathayibacter rathayi* DSM 7485^T (99.65%) and *Rathayibacter iranicus* VKM Ac-1602^T (99.65%). The pairwise average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between VKM Ac-2596^T and VKM Ac-2671 towards the type strains of *Rathayibacter* species did not exceed 85.24% and 29.40%, respectively, that are well below the thresholds for species delineation. The target strains had key chemotaxonomic properties typical of the genus *Rathayibacter*, namely, the DAB-based peptidoglycan, rhamnose and mannose as the predominant sugars and a rhamnomannan in the cell, the major menaquinone MK-10 and fatty acids of iso-anteiso type, with a large proportion of anteiso-15:0. The strains showed clear differences from the recognized *Rathayibacter* species in several phenotypic characteristics, including the difference in the composition of cell wall glycopolymers. Based on the results obtained in this study and the data published previously, we provide a description of a new species, *Rathayibacter tanaceti* sp. nov., with DL-642^T (= VKM Ac-2596^T = LMG 33114^T) as the type strain.

Abbreviations

ANI	Average nucleotide identity
dDDH	Digital DNA-DNA hybridization
DAB	Diaminobutyric acid
DPG	Diphosphatidylglycerol
PG	Phosphatidylglycerol
G	Glycolipids
L	Lipids
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
DDBJ	DNA Data Bank of Japan
ENA	European Nucleotide Archive
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
VKM	All-Russian Collection of Microorganisms

The DDBJ/ENA/GenBank accession numbers for the 16S rRNA gene sequences of strains VKM Ac-2596^T and VKM Ac-2761 are KU891049 and MT431568, respectively. The genome sequences are available at DDBJ/EMBL/GenBank under the accession numbers SLWP01000000 and CP047186.

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Introduction

Bacteria of the genus *Rathayibacter* Zgurskaya et al. 1993 [1] have been known since the end of the last century when Emerich Ráthay revealed a bacterial disease of orchard grass (*Dactylis glomerata* L.) and an associated bacterium (currently *Rathayibacter rathayi*) [2, 3]. For a long time, the first three *Rathayibacter* species discovered

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(*R. rathayi*, *R. tritici*, *R. iranicus*) were affiliated with the chemotaxonomically heterogeneous genus *Corynebacterium* [4]. These species were later transferred to the genus *Clavibacter* established for plant pathogenic bacteria with 2,4-diaminobutyric acid in cell-wall peptidoglycan and some associated characteristics [5]. The genus *Rathayibacter* was proposed to accommodate three *Clavibacter* species (*R. rathayi*, *R. tritici*, *R. iranicus*) that formed a distinct cluster defined by phenetic numerical analysis and differed from *Clavibacter* sensu stricto by the major menaquinone MK-10 and rhamnose and mannose as predominant cell wall sugars [1].

Later on, Sasaki et al. 1998 [6] moved *Clavibacter toxicus* to the genus *Rathayibacter* based on their data that this species and all *Rathayibacter* species have identical l-DAB-based peptidoglycan and formed a common branch on the phylogenetic tree derived from 16S rDNA gene sequences.

At the time of writing, the genus *Rathayibacter* contained nine species with validly published names (https://lpsn.dsmz.de/genus/rathayibacter). In addition, several other putative new species of this genus, including "*Rathayibacter tanaceti*", were revealed but have not yet been validly described [7–10].

The well-known plant pathogenic species (*R. rathayi*, *R. tritici*, *R. iranicus*, and *R. toxicus*) affect wheat and several grasses in the family Poaceae. *R. toxicus* in addition produces a corynetoxin (tunicamycin) in infected ryegrass and some other grasses which is responsible for livestock deaths in Australia [7]. Another plant disease-associated species, *R. agropyri*, was recently described for strains from herbarium material (*Agropyron* sp.) and fresh *Pascopyrum smithii* sample [11]. And finally, *R. festucae* was found in a nematode-induced leaf gall on *Festuca rubra* [12]. The above *Rathayibacter* species were reported to be transferred or can be transferred to their host plants in the family Poaceae by nematodes of the genus *Anguina* (order Tylenchida, family Anguinidae) [7, 11, 12].

The type strains of the remaining *Rathayibacter* species, *R. caricis*, *R. oskolensis* and *R. rubneri*, were isolated from plants of the families Cyperaceae, Primulaceae and Amaryllidaceae, respectively, that had no visible sings of bacterial or nematode infestation [12–14]. These data and the results derived from genomic and metagenomic studies of plant microbiomes demonstrate that rathayibacters may occur as endophytes and phyllosphere inhabitants in a variety of plant species (see Tancos et al. 2021 [15] for references).

Unlike all the recognized *Rathayibacter* species associated with plants known or supposed to be infected by the gall-forming nematodes of the genus *Anguina* (order Tylenchida), two novel strains VKM Ac-2596^T and VKM Ac-2761 characterized in this work were isolated from a plant (*Tanacetum vulgare*) infected by the foliar nematode *Aphelenchoides* sp., order Aphelenchida [9, 10].

Based on the preliminary studies [9, 10], these strains were shown to be genetically unique and deserved description as a novel species of *Rathayibacter.*

Materials and Methods

Isolation, Cultivation, and Maintenance of Strains

The plant samples infested by *Aphelenchoides* sp. were collected in the Main Botanical Garden of the Russian Academy of Sciences, Moscow, Russia. The air-dried leaves were soaked in distilled water for 1 h, washed twice with sterile distilled water, placed in 0.85% NaCl solution, and milled. One drop of the obtained suspension was plated onto Reasoner's 2A (R2A) agar (Fluka Analytical, USA) and incubated for 3 weeks at room temperature (≈ 20–24 °C). The pure cultures were re-isolated on peptone-yeast-glucose (PYG) agar medium (5.0 g peptone, 3.0 g yeast extract, 5.0 g glucose, 0.2 g K_2HPO_4, 15.0 g agar, 1000 ml tap water, pH 7.0) and stored on R2A as well as lyophilized for long term storage.

Analysis of the 16S rRNA Gene and Whole Genome

DNA extraction, PCR and analysis of the 16S rRNA gene sequences of VKM Ac-2596^T and VKM Ac-2761 were performed as described previously [13], except for the 16S rRNA gene sequence identity estimated with TaxonDC (https://tarlachkov.ru/ru/software/taxondc). The draft genome sequence of VKM Ac-2596^T was obtained on the Illumina platform as described [16]. The complete genome sequence of VKM Ac-2761 was generated using Oxford Nanopore and Illumina technologies [10]. Genome quality was assessed by CheckM 1.2.2 (https://github.com/Ecogenomics/CheckM). The genome sequences of VKM Ac-2596^T (SLWP01000000), VKM Ac-2761 (CP047186), and type strains of *Rathayibacter* species available from the NCBI GENOME database (http://www.ncbi.nlm.nih.gov/genome/) were used for comparative genome analyses. The average nucleotide identity (ANI) values were computed using the JSpecies (http://jspecies.ribohost.com/jspeciesws). The digital DNA-DNA hybridization (dDDH) values were calculated with formula two at the Genome-to-Genome Distance Calculation (GGDC) website (http://ggdc.dsmz.de/distcalc2.php). The phylogenomic tree was constructed using JolyTree v2.1.211019ac [17].

Morphological, Physiological and Biochemical Characterization

Morphology and life cycle were observed by phase-contrast microscopy in cultures grown on R2A (Fluka Analytical,

USA) and PYG agar medium. Cells' preparation for SEM was performed according to the scheme described [18], and samples were viewed in JSM-6510LV scanning electron microscope (JEOL, Japan).

Biochemical and physiological tests were performed by conventional methods as reported [1, 13]. Utilization of carbon sources for growth was tested on the mineral Pridham-Gottlieb medium [19] supplemented with 0.05% yeast extract and each carbon source at final concentrations of 1%. Enzyme profile was determined using API ZYM strips (bioMérieux) according to the manufacturer's instructions.

Chemotaxonomic Analysis

Unless otherwise specified, the cells for chemotaxonomic analyses were grown in liquid PYG medium in shake flasks for 36 h at 28 °C, harvested by centrifugation, washed with distilled water and freezed or freeze-dried prior to the analyses. The cell walls were obtained by differential centrifugation after ultrasonic disruption of wet cells and purified with 2% sodium dodecyl sulfate (SDS) (100 °C, 5 min) [20]. Cell wall sugars were analyzed in the acid hydrolysates (3 M trifluoroacetic acid, 100 °C, 4 h) of cell walls as reported [13]. To obtain peptidoglycan, the cell walls were additionally treated with trypsin (1 mg/ml in Tris–HCl buffer, pH 7.85) and 4% SDS (100 °C, 5 min) [21]. The peptidoglycan amino acids were determined by amino acid analyser (Biotronic LC 600) after acid hydrolysis of the sample (6 M HCl, 105 °C, 6 h). Menaquinones were extracted and purified according to Collins and Jones 1980 [21] and analyzed by mass spectrometer LCQ Advantage MAX (Thermo Finnigan). Polar lipids were extracted, separated by two-dimensional TLC and identified as reported [21]. Cellular fatty acids were acquired via saponification, methylation, and extraction and analyzed as recently described [22].

For matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), bacteria were grown on R2A medium at 28 °C for 96 h (4 independent experiments for each strain) and analyzed using mass spectrometer "Autoflex Speed" (Bruker Daltonics, Germany) as described previously [13, 23].

Nucleotide and Genome Sequence Accession Numbers

The DDBJ/ENA/GenBank accession numbers for the 16S rRNA gene sequences of strains VKM Ac-2596^T and VKM Ac-2761 are KU891049 and MT431568, respectively. The genome sequences are available at DDBJ/EMBL/GenBank under the accession numbers SLWP01000000 and CP04718, respectively.

Results and Discussion

The 16S rRNA Gene-Based Phylogeny

Strains VKM Ac-2596^T and VKM Ac-2761 shared 100% 16S rRNA gene sequence identity and formed a separate branch on the *Rathayibacter* phylogenetic trees (Supplementary Figs. S1 and S2). The strains were the most closely related to *Rathayibacter agropyri* CA4^T (99.71%), *Rathayibacter rathayi* DSM 7485^T (99.65%), and *Rathayibacter iranicus* VKM Ac-1602^T (99.65%), showing the minimal relatedness towards *R. toxicus* DSM 7488^T (98.15%).

Genomic Features

The assembled genome sequence of strain VKM Ac-2596^T was found to be ~ 3.2 Mb long, composed of 21 scaffolds with an N50 of 287,715 bp, with a DNA G + C content of 70.8% and coverage of 469×. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) revealed 3 rRNA genes, 47 tRNA genes and 2985 protein-coding genes. The size of assembled genome sequence of strain VKM Ac-2761 was similar, ~ 3.2 Mb, and composed of complete circular chromosome, with a DNA G + C content of 70.7% and coverage of 1111×. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) revealed 6 rRNA genes, 47 tRNA genes and 2932 protein-coding genes. According to the CheckM genomes quality analysis, the completeness of both genomes was 99.41%, and contamination was 2.53%.

The full-length 16S rRNA gene sequences of both strains obtained by the conventional Sanger were 100% identical with the 16S rRNA gene sequence extracted from the whole genome assemblies.

Strains VKM Ac-2596^T and VKM Ac-2671 clearly differed from the type strains of the recognized *Rathayibacter* species in the genome characteristics, including genome size, G + C content and a number of protein-coding genes (Table 1). It is interesting that the sizes, G + C contents and numbers of protein-coding genes were lower in the genomes of *Rathayibacter* species associated with plant diseases than those in the species for which plant pathogenic properties were not registered. In doing so, *R. tanaceti* was adjoined to the plant pathogenic species by its genome size and a number of protein-coding genes.

Both the ANI and dDDH values between VKM Ac-2596^T and VKM Ac-2761 were 100% (Table 1, Supplementary Table S1). The ANI and dDDH values for the above strains towards the type strains of *Rathayibacter* species did not exceed 85.2% and 29.4%, respectively (Table 1, Supplementary Table S1), which are below the

Table 1 Genomic characteristics of *Rathayibacter tanaceti* and type strains of other *Rathayibacter* species

#	Strain	Genome size, Mb	Protein coding genes	GC, %	ANI, %*	dDDH, %*
1	*R. tanaceti* VKM Ac-2596^T (SLWP01000000)	3.19	2931	70.8	–	–
2	*R. tanaceti* VKM Ac-2761 (CP047186)	3.21	2932	70.7	100.00	100.00
3	*R. agropyri* CA-4^T (JABRPL01000000)	3.04	2685	68.1	84.34	28.30
4	*R. tritici* DSM 7486^T (PSWS01000000)	3.17	2957	69.8	85.24	29.40
5	*R. rathayi* VKM Ac-1601^T (WUCA01000000)	3.21	2880	69.3	84.90	29.00
6	*R. iranicus* VKM Ac-1602^T (WUCB01000000)	3.38	2974	67.2	83.81	27.40
7	*R. oskolensis* VKM^T Ac-2121(FXBM01000000)	3.95	3627	71.6	83.13	26.50
8	*R. rubneri* ZW T2_19^T (JAMRYM01000000)	4.01	3741	71.8	82.88	26.50
9	*R. caricis* DSM 15933^T (PZPL01000000)	4.12	3813	71.4	82.80	26.10
10	*R. festucae* DSM 15932^T (CP028137)	4.36	3929	72.3	83.42	27.10
11	*R. toxicus* DSM 7488^T (AUDF01000000)	2.30	2037	61.5	76.61	20.30

*Figures in the columns indicate the ANI and dDDH values between *R. tanaceti* VKM Ac-2596^T and other strains shown in Table

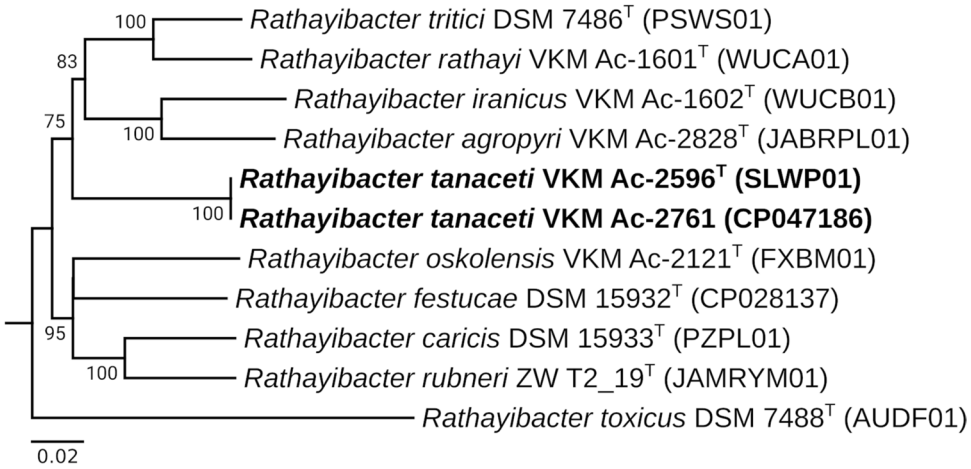

Fig. 1 Phylogenomic tree built using JolyTree v2.1.211019ac (Criscuolo, 2020) showing the position of strains VKM Ac-2596^T and VKM Ac-2761 within the genus *Rathayibacter*. The tree is drawn to scale, with branch lengths measured in the estimated evolutionary distance. Branch support values above 70% are shown at the branch points. The genome sequence of *Clavibacter sepedonicus* ATCC 33113^T (AM849034.1–AM849036.1) served as an outgroup (not shown)

thresholds for delineation of bacterial species [18, 24]. The phylogenomic tree constructed using JolyTree provided further evidence for distinct species position of *R. tanaceti* within the *Rathayibacter* cluster (Fig. 1).

Phenotypic Characteristics

The classical phenotypic properties of strains VKM Ac-2596^T and VKM Ac-2761 (given in the species description and in Table 1) were generally consistent with characteristics of the genus *Rathayibacter*. Cells are Gram-stain-positive, non-motile, irregular rods (0.4–0.6 × 0.8–1.8 µm in young cultures), occurring singly or in pairs with diphtheroid arrangements (Fig. 2).

Testing the antibiotic sensitivity showed that both strains grew in the presence of neomycin (5 mcg); growth was inhibited by ampicillin (30 mcg), chloramphenicol (10 mcg), clindamycin (10 mcg), doxycyclin (10 mcg), gentamicin (30 mcg), kanamycin (5 mzcg), lincomycin (10 mcg), oxacillin

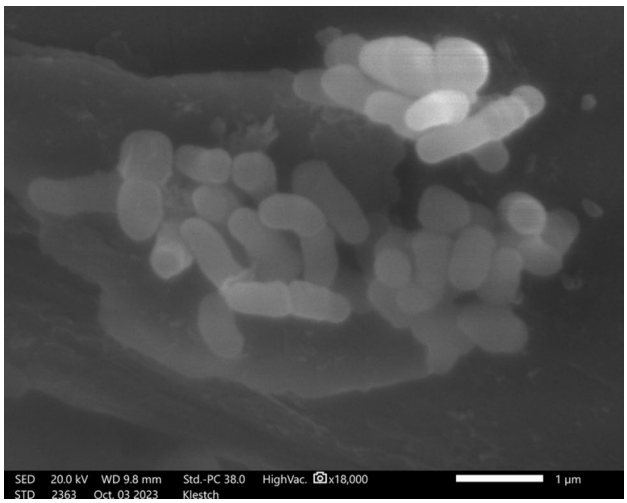

Fig. 2 Scanning electron micrograph of strain VKM Ac-2596^T grown on PYG agar at 28 °C for 2 days

(5 mcg), penicillin (10 mcg), rifampin (30 mcg), streptomycin (10 mcg), and tetracyclin (10 mcg).

The key chemotaxonomic characteristics determined in strain VKM Ac-2596^T were typical of the genus *Rathayibacter* [1, 13, 14]. The cell wall peptidoglycan contained glycine, glutamic acid, 2,4-diaminobuthyric acid and alanine in molar ratios of 1.0: 0.8: 1.7: 1.1 (21.7%: 17.4%: 37.0%: 23.9%). The major menaquinone of strains was MK-10, with minor amounts of MK-9.

The fatty acids (%) of strains VKM Ac-2596^T and VKM Ac-2761 determined in this study were of the iso-anteiso type [25] and included predominant anteiso-15:0 (65.0% and 67.9%) and iso-16:0 (13.5% and 11.5%). Other identified components (> 1%) were iso-17:0 (6.9% and 4.7%), 16:0 (6.3% and 6.0%), 15:0 (4.2% and 4.9%) and anteiso-17:0 (4.1% and 5.2%). The data obtained were in line with the values determined in members of other *Rathayibacter* species in this study (Supplementary material, Table S2). Different values were found in VKM Ac-2596^T in our earlier experiment. Although predominant fatty acid was the same, anteiso-15:0, the relative amount of this acid was lower (41.3%), while values of anteiso-17:0 and some other components increased (Supplementary material, Table S2). This is consistent with the data that fatty acid compositions in bacteria with the iso-anteiso type may vary remarkably depending on the growth conditions [21, 25, our unpublished data].

The polar lipids of strain VKM Ac-2596^T were represented by diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), a few unidentified glycolipids (G1, G2-1, and G2), phospholipids (PL2, PL3-1, and PL6), and also contained lipid (L) not stained by any of the spray reagents (α-naphthol, molybdenum blue, or ninhydrin), except for molybdatophosphoric acid (Fig. 3). In addition, strain VKM Ac-2596^T (and also *R. festucae* VKM Ac-1390^T and *R. oskolensis* VKM Ac-2121^T tested by us under the same conditions [13]) had different unidentified phospholipids that were lacking in *R. rathayi* and *R. iranicus* tested by us previously [13] and by Collins and Jones 1980 [21]. The glycolipids (G1, G2 and G3) detected in VKM Ac-2596^T were not analyzed for chemical structure, but these were identical by their chromatographic behavior and staining to the respective glycolipids (G1, G2 and G3) found in *R. iranicus*, *R. tritici* and some other *Microbacteriaceae* [21]. It is worth noting that glycolipids G2 (according to Collins and Jones 1980 [21]) and G2-1 (revealed in this study in VKM Ac-2596^T and also found in our previous work in *R. festucae* VKM Ac-1390^T and *R. oskolensis* VKM Ac-2121^T [13] although very close to each other, are not identical; they are clearly distinguished by their chromatographic mobilities in some solvent systems (data not presented).

The determined cell wall sugars were rhamnose, mannose, with minor or trace amounts of glucose, galactose and xylose (Table 2). The rhamnose and mannose are suggested to be involved in rhamnomannans, a glycopolymer type revealed in the cell walls of the target strain [26] and most other *Rathayibacter* species studied to date [27–30]. It is worth noting that VKM Ac-2596^T and type strains of other species of this genus are characterized by individual combinations and chemical structures of their cell wall glycopolymers (rhamnomannans, rhamnans and teichuronic acids) (Table 3, Supplementary material, Table S3).

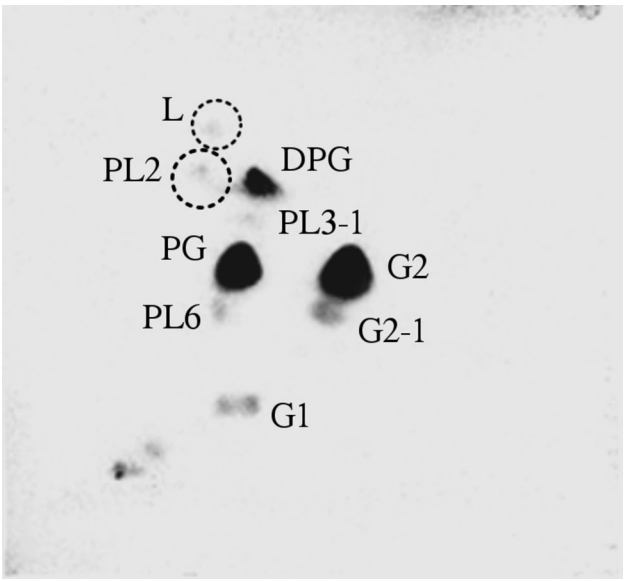

Fig. 3 Polar lipid profile of *Rathayibacter tanaceti* VKM Ac-2596^T after separation by two-dimensional thin layer chromatography and staining with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; L, unidentified lipids; PL, unidentified phospholipids; G, unidentified glycolipids. Designations of glycolipids G1 and G2 according to Collins and Jones 1980 [21] and Dorefeeva et al. 2018 [13]

According to MALDI-TOF mass spectrometry, strains VKM Ac-2596^T and VKM Ac-2761 were grouped into the genus *Rathayibacter*, but clustered separately from the recognized *Rathayibacter* species (Supplementary material, Fig. S3). Several mass-spectral peaks (registered in all spectra obtained for these strains in four different experiments and listed in the species description) were specific for this species. The presence of peaks at m/z 3954, 4428 and 6458 in the spectrum of VKM Ac-2596^T and VKM Ac-2761 were in line the suggestion [13] that these peaks can be considered as chemotaxonomic markers of the genus *Rathayibacter*.

The results of the phenotypical characterization and some selected differentiating properties are summarized in Table 1 in the species description.

Table 2 Phenotypic profiles of *Rathayibacter tanaceti* sp. nov. and other species of the genus *Rathayibacter*

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony color	yellow	yellow	yellow	yellow	yellow	yellow	rose-orange	yellow	yellow	yellow
*Cell wall sugars**										
Fucose	–	–	–	–	–	+	–	–	–	–
Galactose	(+)	(+)	+	(+)	(+)	–	–	+	–	–
Xylose	(+)	+	–	–	+	+	+	+	–	–
Glucose	(+)	+	+	+	+	+	+	+	+	+
Mannose, rhamnose	+	+	+	+	+	+	+	+	+	+
Utilization of										
Adonitol	–	–	–	–	–	+	+	–	–	nd
Dulcitol	–	–	–	–	–	+	+	+	–	nd
Inulin	+	–	+	v	+	+	+	+	–	–
Lyxose	–	–	–	–	–	–	–	+	–	nd
Melibiose	+	–	–	–	–	+	+	+	–	–
l-Rhamnose	+	–	–	–	–	+	+	+	–	nd
Hydrolysis										
Tween 40	+	+	nd	+	+	+	+	–	nd	nd
Tween 60	+	+	nd	v	+	–	+	–	nd	–
Tween 80	–	–	nd	v	v	–	+	–	nd	–
H_2S production	+	+	nd	+	+	+	+	–	nd	–[a]
Growth at 5% NaCl	–	–	nd	–	v	+	–	–	+	–[a]
Source, plant	*Tanacetum vulgare* L	*Dactylis glomerata* L	*Pascopyrum smithii* Rydb	*Triticum aestivum* L	*Triticum aestivum* L	*Carex* sp.	*Festuca rubra* L	*Androsace koso-poljanskii* Ovcz	*Allium cepa* var. Rijnsburger	*Lolium rigidum* Gaud.[a]
Nematode associated	*Aphelenchoides* sp.	*Anguina* sp.	*Anguina* sp.	*Anguina tritici*	*Anguina tritici*	No data	*Anguina graminis*	No data	No data	*Anguina funesta*[a]

Strains: 1, *Rathayibacter tanaceti* VKM Ac-2596^T and VKM Ac-2761; 2, *R. rathayi* VKM Ac-1601^T (type strain of type species of the genus); 3, *R. agropyri* CA-4^T; 4, *R. iranicus* VKM Ac-1602^T; 5, *R. tritici* VKM Ac-1603^T; 6, *R. caricis* VKM Ac-1799^T; 7, *R. festucae* VKM Ac-1390^T; 8, *R. oskolensis* VKM Ac-2121^T; 9, *R. rubneri* ZW T2_19^T; 10, *R. toxicus* DSM 7488^T. Data from this study and from Zgurskaya et al. 1993 [1], Dorofeeva et al. 2002 [12] and Dorofeeva et al. 2018 [13], except for data on *R. agropyri* CA-4^T and *R. rubneri* ZW T2_19^T taken from Schroeder et al. 2018 [11] and Stoll et al. 2023 [14]. Cell wall sugars and other data on *R. toxicus* are given for the non-type strain, VKM Ac-1600, unless indicated; [a] data from Sasaki et al. 1993 [6]. +, Positive; –, negative; v, variable between experiments or test methods; nd, no data. Sugars in parentheses were detected in minor or race amounts or varied between experiments (Dorofeeva et al. 2002 [12]; Dorofeeva et al. 2018 [13])

*Sugar pattern for *R. rubneri* are from whole cells and includes additionally ribose

Table 3 The cell wall glycopolymers of *Rathayibacter* species

Strain	Cell wall glycopolymers and components of their main-chains and lateral substituents (in square brackets)				References
	Neutral glycopolymers		Acidic glycopolymers (teichuronic acids)		
R. tanaceti sp. nov. VKM Ac-2596^T	RM 1	d-Rha, d-Man	–	–	Shashkov et al., 2018 [27]
R. toxicus VKM Ac-1600	RM 2	d-Rha, d-Man	–	–	Shashkov et al., 2018 [27]
	GM 1	d-Glc, d-Man [l-Rha]			
R. iranicus VKM Ac-1602^T	RM 3	d-Rha, d-Man [d-Man]	TUA 1	Glc*p*A, l-Rha, d-Man, d-Gal, d-Glc, [d-Glc]	Dmitrenok et al., 2019 [28]
R. tritici VKM Ac-1603^T	RM 4	d-Rha, d-Man	TUA 2	Glc*p*A, d-Man, d-Glc, d-Gal, [d-Glc]	Shashkov et al., 2020 [26]
	RM 5	d-Rha, d-Man [d-Xyl]			
R. caricis VKM Ac-1799^T	R 1	d-Rha [d-Ara, d-Man]	TUA 3	Glc*p*A, d-Man, d-Glc, l-Rha, 4,6-Pyr, [d-Man]	Shashkov et al., 2021 [27]
R. festucae VKM Ac-1390^T	RM a	d-Rha, d-Man [nd]	TUA d	nd	Potekhina et al., 2023 [30]
R. oskolensis VKM Ac-2121^T	RM b	d-Rha, d-Man [nd]	TUA e	nd	Potekhina et al., 2023 [30]
R. rathayi VKM Ac-1601^T	RM c	d-Rha, d-Man [nd]	TUA f	nd	Potekhina et al., 2023 [30]
R. agropyri CA-4^T	RM or R	Rha, Man or Rha [nd]*	nd	nd	Schroeder et al., 2018 [11]
R. rubneri ZW T2_19^T	RM or R	Rha, Man or Rha [nd]*	nd	nd	Stoll et al., 2023 [14]

R, rhamnan; RM, rhamnomannan; GM, glucomannan; TUA, teichuronic acid; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Xyl, xylose; Glc*p*A, glucuronic acid; 4,6-Pyr, pyruvic acid; nd, no data. Numerals at R, RM and TUA indicate different structures of respective polymers; for more details, see Table S3 (Supplementary material) and original publications. Letters a-f indicate partially identified glycopolymers structurally different from each other and from the identified polymers (preliminary data of Streshinskaya et al. 2016 [29])

*The presence of rhamnomannans and/or rhamnans in *R. agropyri* and *R. rubneri* are assumed on the basis of predominant mannose and rhamnose in the cell wall or whole cell sugars (Schroeder et al. 2018 [11]; Stoll et al. 2023 [14])

Conclusion

With the consideration of clear genotypic and phenotypic differences between new strains VKM Ac-2596^T and VKM Ac-2761, and the recognized *Rathayibacter* species, including the difference in the cell wall glycopolymers, we provide the description of a novel species with the name *Rathayibacter tanaceti* sp. nov.

Description of *Rathayibacter tanaceti* sp. nov.

Rathayibacter tanaceti [ta.na.ce'ti N.L. gen. n. *tanaceti* referring to *Tanacetum*, tansy, the generic name of the plant from which the type strain was isolated].

Cells are Gram-stain-positive, non-motile, irregular rods (0.4–0.6 × 0.8–1.8 µm in young cultures), occurring singly or in pairs with diphtheroid arrangements. Primary branching was not observed. In older (2–5 day) cultures, coccoid and coccobacillar forms predominant; occur singly or in pairs, short chains or clumps. A marked life cycle could be observed on peptone-yeast-glucose (PYG) agar and on brain heart infusion agar (Conda, Spain). Colonies grown on R2A and PYG agar are yellow-pigmented, circular, slightly convex, entire, opaque and butyrous.

Aerobic. Catalase-positive. Oxidase test reaction with tetramethyl-*p*-phenylenediamine is negative or weak. Cells are capable of producing leucine arylamidase, no other positive reactions are observed using API ZYM. Mesophilic; optimum growth temperature is 24–28 °C, no growth at 7 or 37 °C. The pH range for growth of strains was 5.5–9.0 (optimum pH 7.0–8.0). Strains exhibited growth with NaCl concentrations of 1%; no growth was observed at 5% NaCl (w/v) or higher. No growth was observed under anaerobic conditions. d-Fructose, d-galactose, d-glucose, d-xylose, *meso*-inositol, inuline, maltose, mannitol, mannose, melibiose, l-rhamnose, raffinose, salicin, sucrose, trehalose, and turanose are used as carbon sources for growth in a mineral salt medium supplemented with 0.05% (w/v) of yeast extract. Adonitol, dextran, dulcitol, *meso*-erytritol, sorbitol, l-arabinose, lyxose, melicitose, ribose and sorbose are not used as C-source in the same medium. Tween 40 and Tween 60 are hydrolyzed. Tween 80 and casein are not hydrolyzed. H_2S is produced from peptone.

The cell wall peptidoglycan is of B-type based on 2,4-diaminobutyric acid. The cell wall includes a linear rhamnomannan based on d-rhamnose and d-mannose; the predominant components of the cell wall sugar pattern are also rhamnose and mannose. The major menaquinone is MK-10, with minor amounts of MK-9. Fatty acids of iso-anteiso type, with a large proportion of anteiso-15:0. The major polar lipids are diphosphatidylglycerol,

phosphatidylglycerol, and unidentified glycolipid, along with minor amounts of other unidentified components.

The MALDI mass-spectrum of the type strain typically includes the following unique peaks (m/z): 2010, 2470, 2899, 3015, 3046, 4420, 5443, 5797, 5824, 5860, 6031, 6093, 6836, 11,290.

The type strain is DL-642^T (= VKM Ac-2596^T = LMG 33114^T) was isolated from a plant *Tanacetum vulgare* (the family Asteraceae) infested by foliar nematode *Aphelenchoides* sp. (family Aphelenchoididae), that was sampled in the Main Botanical Garden of the Russian Academy of Sciences, Moscow, Russia.

The DNA G + C content of the genome of the type strain is 70.8%, an approximate genome size is 3.2 Mbp. The GenBank accession numbers of the 16S rRNA gene and draft genome sequences of the type strain are KU891049 and SLWP01000000, respectively.

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Data Availability All authors have declared that all data are availability.

Declarations

Conflict of interest The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All the authors declare that they have no conflict of interest.

Ethical Approval The authors have declared that no ethical issues exist.

Research Involving Human and Animal Participants This article does not contain any studies with human participants or animals performed by any of the author.

Consent to Participate All authors agree to have participated in the research proposed to be published.

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