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Rathayibacter rubneri sp. nov. isolated from *Allium cepa* var. Rijnsburger, an onion landrace

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Abstract

The novel, aerobic, Gram-stain-positive, rod-shaped bacterial strain, ZW T2_19^T, was isolated from an onion sample (*Allium cepa* var. Rijnsburger). Analyses of the 16S rRNA gene sequence revealed that ZW T2_19^T represented a member of the genus *Rathayibacter* but may represent a novel species of this genus. Analyses of the whole draft genome sequences, i.e. digital DNA–DNA hybridisation (dDDH) and average nucleotide identity (ANI) of ZW T2_19^T and all type strains of species of the genus *Rathayibacter* confirmed that ZW T2_19^T represents a novel species of the genus *Rathayibacter*. The genome size of ZW T2_19^T is 4.01 Mbp and the DNA G+C content is 71.8 mol%. Glucose, mannose, rhamnose and ribose were detected as whole-cell sugars of ZW T2_19^T. The major respiratory quinone of ZW T2_19^T is menaquinone MK-10, at 78.9%. The detected peptidoglycan type in ZW T2_19^T is a variant of type B2γ with {Gly} [L-diaminobutyric acid (L-DAB)/L-homoserine (L-Hse)] D-Glu-L-DAB. Polar lipids in ZW T2_19^T consisted of one diphosphatidylglycerol, one phosphatidylglycerol, seven glycolipids, one phospholipid and one lipid. The fatty acid profile of ZW T2_19^T predominantly consisted of anteiso-$C_{15:0}$ (53%), iso-$C_{16:0}$ (21%) and anteiso-$C_{17:0}$ (18%). In addition, API 20NE, API 50CH, API Coryne, API ZYM, antibiotic susceptibility, haemolysis and growth at different temperatures and with different supplements was investigated. On the basis of the results obtained using this polyphasic approach, including molecular, phenotypic and biochemical analyses, we propose the novel species *Rathayibacter rubneri* with the type and only strain ZW T2_19^T (= DSM 114294^T = LMG 32700^T).

In 1993, the genus *Rathayibacter* was proposed in honour of Emerich Ráthay, a plant pathologist, who investigated an infection of *Dactylis glomerata* in 1899 caused by bacteria which were later named *Rathayibacter* [1, 2]. The genus *Rathayibacter* is part of the family *Microbacteriaceae* within the class *Actinobacteria* (Phylum *Actinomycetota*). At time of writing, the genus *Rathayibacter* includes the following species, *Rathayibacter agropyri* [3], *Rathayibacter caricis* [4], *Rathayibacter festucae* [4], *Rathayibacter iranicus* [2], *Rathayibacter oskolensis* [5], *Rathayibacter rathayi* (type species of the genus) [2], '*Rathayibacter tanaceti*' [6], *Rathayibacter toxicus* [7] and *Rathayibacter tritici* [2]. It has to be noted that all listed species have validly published names except for '*R. tanaceti*'.

All members of the genus *Rathayibacter* have been isolated from plants, mostly from grasses: Six strains of the nine described species were isolated from plants belonging to the family Poaceae. Some members have been described as being associated with plant diseases, e.g. *R. toxicus* has been described as being associated with annual ryegrass toxicity [8]. Initially, the genus *Rathayibacter* was suggested for the plant pathogenic species *R. tritici*, *R. rathayi* and *R. iranicus* [2] which formerly were members of the genus *Clavibacter* [4, 9]. In addition, *R. festucae* was isolated from a leaf gall of *Festuca rubra* (red fescue) [4]. The leaf gall was induced by *Anguina graminis*, a nematode transferring bacterial strains of *R. festucae* into the plant. In addition, the transfer of *R. rathayi, R. tritici, R. iranicus* and *R. toxicus* by species of the genus *Anguina* into plants has also been reported [4, 10–14].

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Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridisation; D-Glu, D-glutamic acid; GBDP, genome BLAST distance phylogeny; L-DAB, L-diaminobutyric acid; L-Hse, L-homoserine; MALDI-TOF, matrix assisted laser desorption ionisation - time of flight; MK, menaquinone; OrthoANIu, orthologous ANI using USEARCH; SEM, scanning electron microscopy; TYGS, type (Strain) genome server.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain ZW T2_19^T (= DSM 114294^T = LMG 32700^T) is ON892742.
The whole genome shotgun project of strain ZW T2_19^T has been deposited at GenBank/EMBL/DDBJ under the genome accession number JAMRYM000000000.
Three supplementary figures are available with the online version of this article.

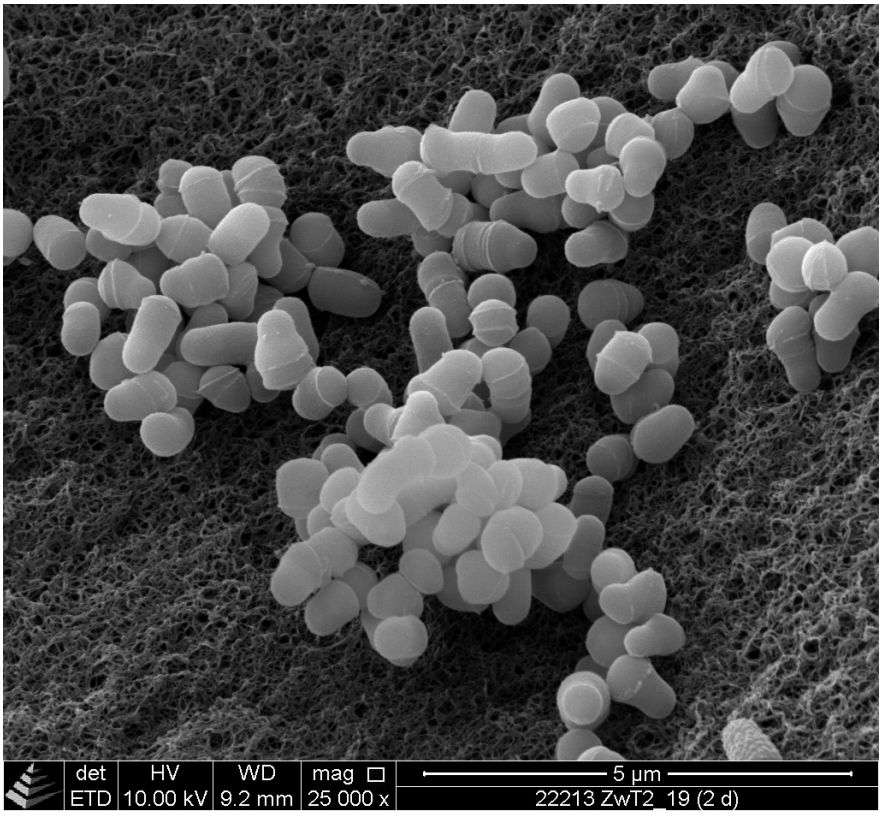

Fig. 1. Scanning electron micrograph of cells of ZW T2_19^T. Bacterial cells were grown on Standard Nutrient I agar for 48 h. Bar, 5 µm.

Strain ZW T2_19^T was isolated during a storage trial in November 2020 which aimed to investigate possible differences in the composition of the microbiota and differences in shelf life of onion landraces versus modern hybrid races. During this storage trial a total of 317 bacterial isolates were collected and identified using 16S rRNA gene sequencing as well as MALDI-TOF analysis. Among this set of strains, 13 isolates, including strain ZW T2_19^T, showed similarity values above the threshold of 94.5% for genus delineation and below the threshold for species identification of 98.7% [15] and could not be identified using MALDI-TOF analysis. Therefore, whole-genome shotgun sequencing was conducted for this subset of 13 bacterial isolates which revealed that ZW T2_19^T may represent a novel species of the genus *Rathayibacter* while the other strains represented members of different genera.

For the isolation of bacterial strains, about a quarter of an onion bulb, including the outer layer, was minced and diluted in quarter-strength Ringer`s solution (Merck) in tenfold serial dilutions and incubated on Standard Nutrient I agar (Merck) at 30 °C. All bacterial isolates were repeatedly streaked out until purity and cryopreserved with 15% (v/v) glycerol (Roth). ZW T2_19^T was isolated from a landrace onion (*Allium cepa* var. Rijnsburger) which was harvested, air-dried and then stored for 2 months at 2 °C and at a humidity of 61%. After 48–72 h, ZW T2_19^T occurred as small, smooth, round and yellow colonies with a diameter of about 2 mm. Mid-exponential to stationary phase cells were visualised under a phase-contrast microscope (Leica) and occurred as non-motile, small rods.

The rod-shaped morphology was confirmed by scanning electron microscopy (SEM) as described previously [16] (Fig. 1). For SEM, bacterial cells were grown on Standard Nutrient I agar for 48 h. Furthermore, the cell diameters and cell lengths of 50 cells of ZW T2_19^T were measured using ObjectJ in ImageJ 1.53q [17]. The mean diameter and mean cell length of ZW T2_19^T were 0.46±0.07 µm and 0.79±0,15 µm, respectively. This corresponds well to the cell width of 0.4 to 0.8 µm and cell length of 0.5 to 1.8 µm as given in the description of the genus *Rathayibacter* [2].

For 16S rRNA gene sequencing, isolation of bacterial DNA of ZW T2_19^T, 16S rRNA gene amplification and analysis were carried out as described previously [18]. Briefly, the bacterial DNA was isolated using a Blood and Tissue Kit (Qiagen) and the almost complete 16S rRNA gene was amplified using the primers 16Sseq fw (5′-ATA GTT TGA TCM TGG CTC AG-3′) and 16Sseq rev (5′-GGN TAC CTT GTT ACG ACT TC-3′). The 16S rRNA gene sequence (1130 bp, Genbank accession number ON892742) of ZW T2_19^T was used for BLASTn search. ZW T2_19^T was classified as representing a member of the genus *Rathayibacter*.

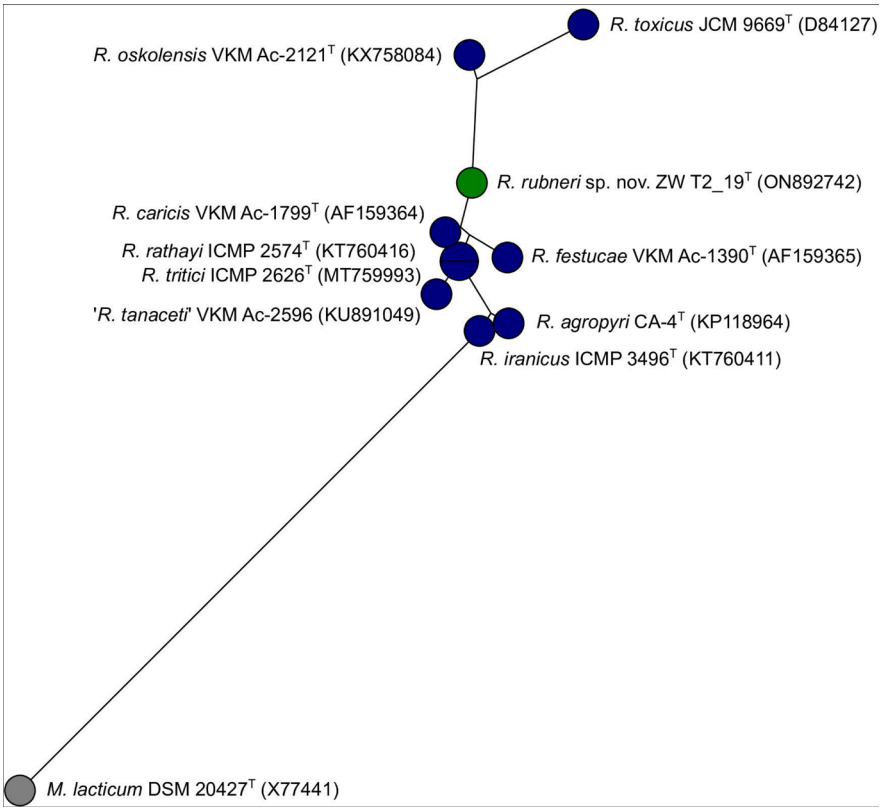

Fig. 2. Analysis of 16S rRNA gene sequences of ZW T2_19^T and all nine type strains of species of the genus *Rathayibacter*. *Microbacterium lacticum* DSM 20427^T was used as an outgroup. The tree was reconstructed using maximum-likelihood with Jukes–Cantor as the evolutionary model (BioNumerics, version 8.1; Applied Maths).

BioNumerics (version 8.1, Applied Maths) was used to calculate similarity values of pairwise comparisons with the nearest neighbours. The 16S rRNA gene sequence of ZW T2_19^T showed similarity values of 98.7, 98.6 and 97.5% to *R. caricis* VKM Ac-1799^T, *R. rathayi* VKM Ac-1601^T and *R. festucae* DSM 15932^T, respectively. These results indicated that all similarity values were below the threshold for species differentiation based on 16S rRNA gene sequences [15].

Fig. 2 shows a maximum likelihood tree (BioNumerics) based on the 16S rRNA gene sequences of type strains of species of the genus *Rathayibacter*. The nearest neighbour of ZW T2_19^T was the type strain *R. caricis* VKM Ac-1799^T. However, ZW T2_19^T was clearly separated from the nearest related type strains of species of the genus *Rathayibacter* (Fig. 2). The cluster analysis was repeated with two additional clustering methods, namely neighbour joining and maximum parsimony, and confirmed the taxonomic position of ZW T2_19^T (Figs S1 and S2, available in the online version of this article).

The whole draft genome sequence of ZW T2_19^T was sequenced on an Illumina MiSeq by our group and has been deposited at GenBank/EMBL/DDBJ under the genome accession number JAMRYM000000000. Characteristics of the genome sequences of ZW T2_19^T and all nine validly or effectively published type strains of species of the genus *Rathayibacter* [e.g. genome size, DNA G+C content (mol%) and number of proteins] were obtained using the Type (Strain) Genome Server (TYGS) [19] and are summarised in Table 1. The genome characteristics of ZW T2_19^T, with a length of 4.01 Mbp, 71.8mol% DNA G+C content and 3848 proteins, were similar to those of the type strains of species of the genus *Rathayibacter*, as the genome sizes, DNA G+C contents and numbers of proteins of the type strains of species of the genus *Rathayibacter* ranged from 2.29 to 4.36 Mbp, 61.5 to 72.3 mol% and 2202 to 3952 proteins, respectively. In addition, the results of digital DNA–DNA hybridisation (dDDH) calculated by using TYGS [19, 20] and average nucleotide identity (ANI) as orthologous ANI using USEARCH (OrthoANIu) values calculated by using EzBioCloud [21, 22] are included in Table 1. The dDDH values of ZW T2_19^T compared with the type strains of species of the genus *Rathayibacter* were clearly below the threshold of 70% for species-delimitation [23, 24] with the highest similarity of ZW T2_19^T to the type strain *R. caricis* with a dDDH value of 36.7% (Table 1). All OrthoANIu values of ZW T2_19^T compared with the type strains of species of the genus *Rathayibacter* were below the 95–96% cut-off value for species delimitation [25] and similar to the results for dDDH: The highest similarity compared with ZW T2_19^T was observed for the type strain of *R. caricis* with a value of 88.82%. A Genome BLAST Distance Phylogeny (GBDP) tree (whole-genome sequence-based)

Table 1. Whole draft genome characteristics, values of digital DNA–DNA hybridisation (dDDH) and average nucleotide identity (ANI) of ZW T2_19^T and strains of members of the genus *Rathayibacter*

Strains: 1, *R. rubneri* sp. nov. ZW T2_19^T (GCF_023743255); 2, *R. agropyri* VKM Ac-2828 (GCF_013249015); 3, *R. caricis* DSM 15933^T (GCF_003044275); 4, *R. festucae* DSM 15932^T (GCF_004011135); 5, *R. iranicus* DSM 7484^T (GCF_002933035); 6, *R. oskolensis* VKM Ac-2121^T (GCF_900177245); 7, *R. rathayi* DSM 7485^T (GCF_004011095); 8, '*R. tanaceti*' VKM Ac-2596 (GCF_004340625); 9, *R. toxicus* DSM 7488^T (GCF_000425325); 10, *R. tritici* DSM 7486^T (GCF_002932875).

Characteristic	1	2	3	4	5	6	7	8	9	10
Genome length (Mbp)*	4.01	3.04	4.12	4.36	3.39	3.95	3.10	3.19	2.29	3.17
DNA G+C content (mol%)*	71.8	68.1	71.4	72.3	67.2	71.6	69.4	70.8	61.5	69.8
Number of proteins*	3848	2903	3854	3952	3280	3668	2992	2987	2202	3012
dDDH (%)* versus ZW T2_19^T	–	25.2	36.7	28.2	24.7	27.8	25.6	26.5	20.8	25.9
ANI (%)† versus ZW T2_19^T	–	82.31	88.82	84.24	81.99	83.97	82.52	83.13	77.09	82.75

*Values were obtained from TYGS [19, 20]. For dDDH formula d4 is given
†Values were obtained from EzBioCloud [21, 22]

of strain ZW T2_19^T and type strains of species of the genus *Rathayibacter* was calculated by using TYGS [19]. Consistently with the results for dDDH and ANI, the nearest neighbour of ZW T2_19^T was the type strain of *R. caricis* DSM 15933^T (Fig. 3).

Traitar was used for the *in-silico* prediction of the bacterial phenotype of ZW T2_19^T on the basis of the genome sequence [26]. Phenotypic characteristics according to both predictors implemented in Traitar are Gram-stain-positive, catalase- and coagulase- positive, aerobic bacilli or coccobacilli. ZW T2_19^T is able to grow on ordinary blood agar as well as in 6.5% NaCl, however, the cells are bile-susceptible. Voges–Proskauer and ONPG (β-galactosidase) positive test reactions were predicted. The strain was predicted to be able to utilise the carbohydrates L-arabinose, cellobiose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, L-rhamnose, sucrose, trehalose and xylose. The predicted phenotype of ZW T2_19^T also showed an alkaline phosphatase activity and the ability to hydrolyse aesculin; in addition the strain was predicted to be able to utilise citrate and

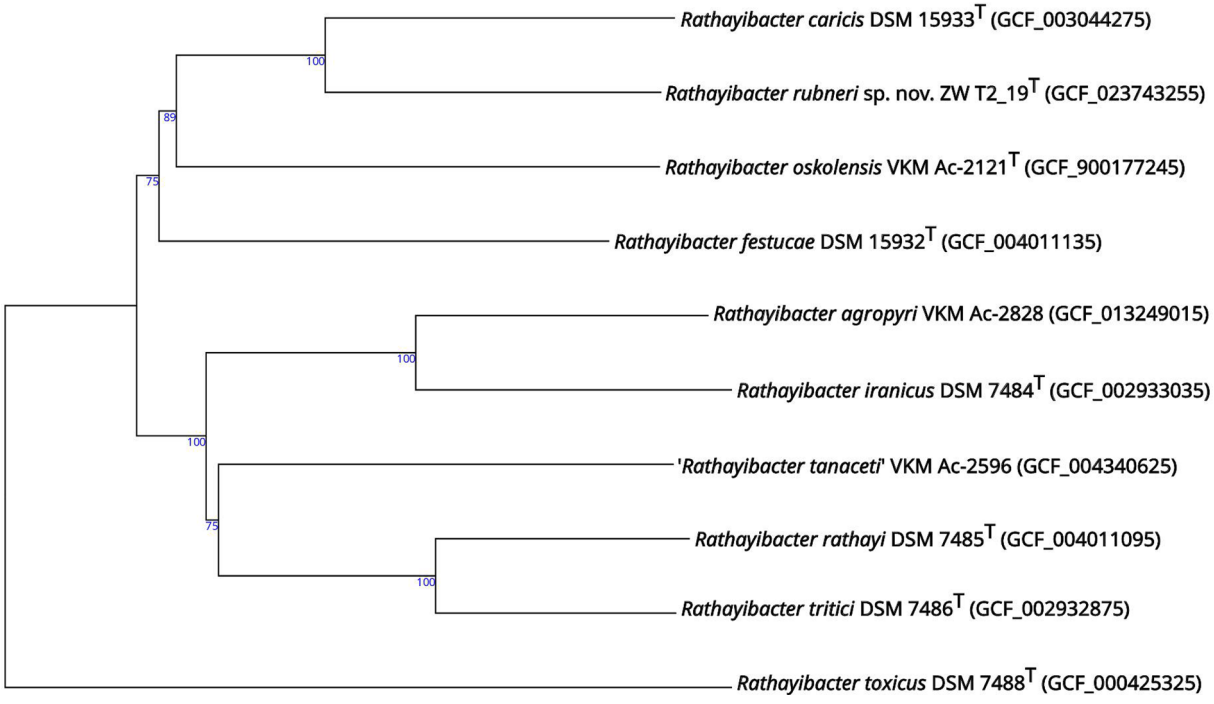

Fig. 3. Genome BLAST Distance Phylogeny (GBDP) tree (whole-genome sequence-based) of ZW T2_19^T and strains of members of the genus *Rathayibacter*. The tree was reconstructed using the type strain genome server (TYGS) [19]. Tree inferred with FastME 2.1.6.1 [40] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values >60% from 100 replications, with an average branch support of 91.3%. The tree was rooted at the midpoint [41].

Table 2. Phenotypic and biochemical characteristics of strains of members of the genus *Rathayibacter*

+, Positive reaction; −, negative reaction; (+), reaction variable among strains; w, weak or negative; ND, not detected; TR, traces; L-DAB, L-diaminobutyric acid; DPG, diphosphatidylglycerol; PG, phosphatidylglycercol; GL, glycolipid; PL, phospholipid; L, lipid. Strains: 1, *R. rubneri* sp. nov. ZW T2_19^T; 2, *R. agropyri* CA-4^T; 3, *R. caricis* VKM Ac-1799^T; 4, *R. festucae* VKM Ac-1390^T; 5, *R. iranicus* VKM AC-1602^T; 6, *R. oskolensis* VKM Ac-2121^T; 7, *R. rathayi* VKM Ac-1601^T; 8, *R. toxicus* VKM Ac-1600^T; 9, *R. tritici* VKM Ac-1603^T.

Strain	1	2	3	4	5	6	7	8	9
Oxidase	+	−*	w†	+†	w†	w‡	w†	w†	w†
Growth at/with									
7 °C	−	ND*	−†	−†	ND§	−‡	ND§	ND‖	ND§
37 °C	−	ND*	−†	−†	−§	−‡	−§	−‖	−§
42 °C	−	ND*	ND†	ND†	ND§	ND‡	ND§	ND‖	ND§
0.01% KTe	−	ND*	ND†	ND†	+§	ND‡	+§	ND¶‖	+§
0.02% KTe	−	ND*	ND†	ND†	−§	ND‡	−§	ND¶‖	−§
0.03% KTe	−	ND*	−†	−†	+§	ND‡	(+)§	ND¶‖	+§
5% NaCl	+	ND*	w†	−†	−§	−‡	−§	ND¶‖	+§
Whole-cell sugars									
Glucose	+	+*	+†	+†	+†	+‡	+†	+†	+†
Mannose	+	+*	+†	+†	+†	+‡	+†	+†	+†
Rhamnose	+	+*	+†	+†	+†	+‡	+†	+†	+†
Galactose	−	+*	−†	−†	+§/−†	+‡	+†	−†	+†
Xylose	−	−*	+†	+†	−§†	+‡	+†	−†	+†
Fucose	−	−*	+†	−†	−†	−‡	−†	−†	−†
Ribose	+	−*	−†	−†	−†	−‡	−†	−†	−†
Respiratory quinones									
MK8	ND	ND*	+†	+†	TR¶	ND‡	TR¶	TR¶	ND¶
MK9	6.4%	14%*	+†	+†	18%¶	+‡	15%¶	8%¶	10%¶
MK10	78.9%	67%*	predominant†	predominant†	75%¶	predominant‡	74%¶	73%¶	83%¶
MK11	14.7%	6%*	+†	+†	TR¶	ND‡	6%¶	18%¶	7%¶
Peptidoglycan type	Variant of B2γ with L-DAB	B2γ with DAB*	B2γ with DAB†	B2γ with DAB†	B2γ with L-DAB¶§	Of the B group based on 2,4-diaminobutyric acid‡	B2γ with L-DAB¶§	B2γ with L-DAB¶	B2γ with L-DAB¶§
Polar lipids	1 DPG, 1 PG, 7 GL, 1 PL, 1 L	DPG, PG, GL*	DPD, PG†	DPD, PG†	DPG, PG§	DPG, PG, a few GL, a few PL, L‡	DPG, PG§	ND‖	DPG, PG§

*Data obtained from Schroeder *et al.*, 2018 [3].
†Data obtained from Dorofeeva *et al.*, 2002 [4].
‡Data obtained from Dorofeeva *et al.*, 2018 [5].
§Data obtained from Zgurskaya *et al.*, 1993 [2].
‖Data obtained from Riley and Ophel, 1992 [8].
¶Data obtained from Sasaki *et al.*, 1998 [7].

myo-inositol. Additional phenotypic characteristics according to one predictor were oxidase-positivity and ability to grow in KCN, a yellow pigment was predicted to be produced. Further substrates that were predicted to be utilised were acetate, arginine, gelatin, malonate, salicin and starch.

The prediction of the phenotype on the basis of the genome sequence predicted the following traits: Gram-stain-positive, catalase-positive, aerobic with rod-shaped morphology and yellow pigmentation which was confirmed by routine microbiological testing of the bacterial culture of ZW T2_19^T. This corresponds well with the literature as it is stated in the genus description of *Rathayibacter* that strains of this genus are Gram-stain-positive and catalase-positive [2], whereas the oxidase reaction is variable among the species (Table 2). Growth was tested in Standard Nutrient I broth (Sigma-Aldrich Chemie) for up to 7 days at 7, 24, 30, 37 and 42 °C, with supplementation with potassium tellurite (0.01, 0.02 and 0.03%, 24 °C, Merck) and with 5% NaCl (24 °C) and 6.5% NaCl (24 °C). ZW T2_19^T did not grow at 7, 37 or 42 °C, which has also not been described for any of the species of the genus *Rathayibacter*. The comparison of the phenotype based on the genome sequence predicted that ZW T2_19^T is able to grow with 6.5% NaCl but not at 42 °C, which was confirmed by testing the bacterial culture. ZW T2_19^T did not grow with any concentration of potassium tellurite and could be differentiated with respect to this trait from *R. iranicus, R. rathayi* and *R. tritici* (Table 2). However, growth of ZW T2_19^T was detected in Standard Nutrient I broth with 5% NaCl and with 6.5% NaCl at 24 °C after 48 h which has only been described for *R. tritici*. In addition, no growth of ZW T2_19^T was observed in Standard Nutrient I broth supplemented with 1% ox bile (Merck) and testing of gas formation in Standard Nutrient I broth with Durham tubes gave negative results, as predicted by bioinformatic analyses based on the genome sequence. The optimal growth conditions for ZW T2_19^T in Standard Nutrient I broth were 24 °C on a rotary shaker at 100 r.p.m.

Biochemical characteristics of ZW T2_19^T were determined using API 20NE, API 50CH, API Coryne and API ZYM strips (bioMérieux) according to the manufacturer's instructions. Haemolysis was investigated on blood agar (bioMérieux). ZW T2_19^T displays γ-haemolysis, however, it grows well on blood agar as predicted on the basis of the genome sequence. Detailed test results are listed in the species description. Compared with the type strains of the other species of the genus *Rathayibacter* with validly published names and with the phenotype prediction based on the genome sequence, ZW T2_19^T shows a limited ability to utilise different carbohydrates, i.e. mannitol, aesculin and glucose. The majority of the type strains of species of the genus *Rathayibacter* are able to metabolise, for example, arabinose, xylose, galactose, mannose, mannitol, sorbitol and lactose. Susceptibility against the following antimicrobial agents was tested using the EUCAST disc method according to version 10.0 of the manual (January 2022) on Mueller Hinton agar (Fisher Scientific): amikacin (30 µg), ampicillin (10 µg), ampicillin and sulbactam (10 and 10 µg), aztreonam (30 µg), cefepime (30 µg), cefotaxime (5 µg), cefoxitin (30 µg), cefpodoxime (10 µg), ceftazidime (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), linezolid (10 µg), meropenem (10 µg), piperacillin (30 µg), piperacillin and tazobactam (30 and 6 µg, respectively), tetracycline (30 µg) and trimethoprim and sulfamethoxazole (1.25 and 23.75 µg, respectively). No inhibition zones could only be detected in the case of aztreonam and ceftazidime, indicating a distinctive resistance of ZW T2_19^T against these two antimicrobial agents, while clear inhibition zones were observed for the remaining antimicrobial agents. However, according to ResFinder (version 4.1) [27, 28] no antibiotic resistance was detected in the genome of ZW T2_19^T.

Biochemical analyses of ZW T2_19^T were carried out by the Identification Service of the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) including analysis of respiratory quinones [29, 30], fatty acids [31, 32], whole-cell sugars [33, 34], peptidoglycan structure [33, 35–37] and polar lipids [38, 39]. For this purpose, the biomass of 10 l liquid culture (Standard Nutrient I broth, 24 °C, 110 r.p.m., 24 h) of ZW T2_19^T was collected by centrifugation (9622***g***; 10 min; 4 °C) and the total moist biomass of ZW T2_19^T (6.06 g) was sent on dry ice to the DSMZ. Glucose, mannose, rhamnose and ribose were detected as whole-cell sugars of ZW T2_19^T. Glucose, mannose and rhamnose have been described to occur in all species of the genus *Rathayibacter* so far described as cell wall sugars Table 2. The presence of ribose, which was detected in ZW T2_19^T, is described for the first time, to our knowledge, for a member of the genus *Rathayibacter*.

The following respiratory quinones were detected in ZW T2_19^T (Table 2): MK-9 (menaquinone; 6.4%), MK-10 (78.9%) and MK-11 (14.7%). A predominance of MK-10 is a key characteristic of the members of the genus *Rathayibacter* [2]. Furthermore, the presence of MK-9 and MK-11 has been described for the majority of type strains of species of the genus *Rathayibacter* (Table 2). Another key characteristic of the members of the genus *Rathayibacter* is peptidoglycan based on 2,4-diaminobutyric acid (type B2γ) [2] (Table 2) with presence of the L-enantiomer of diaminobutyric acid (DAB) [7]. The detected peptidoglycan type in ZW T2_19^T is a variant of type B2γ with {Gly} [L-DAB/L-homoserine L-Hse] D-Glu-L-DAB. Polar lipids in ZW T2_19^T consisted of one diphosphatidylglycerol, one phosphatidylglycerol, seven glycolipids, one phospholipid and one lipid (Fig. S3). Zgurskaya *et al*. described the presence of phosphatidylglycerol and diphosphatidylglycerol as basic polar lipids in the members of the genus *Rathayibacter* [2] which have been detected in every type strain of species of the genus *Rathayibacter* including strain ZW T2_19^T (Table 2). The fatty acid profile of ZW T2_19^T predominantly consisted of anteiso-$C_{15:0}$ (53%), iso-$C_{16:0}$ (21%) and anteiso-$C_{17:0}$ (18%). These results correspond well to the fatty acid profile description of the type strains of the species of the genus *Rathayibacter* which consists predominantly of anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ and iso-$C_{16:0}$ [2, 5].

On the basis of the results of this study, strain ZW T2_19^T represents the type strain of a novel species within the genus *Rathayibacter*, for which the name *Rathayibacter rubneri* sp. nov. is proposed.

DESCRIPTION OF *RATHAYIBACTER RUBNERI* SP. NOV.

R. rubneri (rub´ne.ri. N.L. gen. n. *rubneri* referring to Max Rubner, a German medical doctor after whom the Max Rubner-Institute was named, where the type strain was isolated).

Cells occur as non-motile, Gram-stain positive and small, single rods. Mean size of cells is 0.79 µm in length and 0.46 µm in width. Colonies (diameter approximately 2 mm) are small, smooth, round and yellow after 48–72 h incubation on Standard Nutrient I agar at 24 °C. Oxidase-positive and catalase-positive. Cells are capable of producing α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, alkaline phosphatase, pyrazinamidase, pyrrolidonylarylamidase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Cells are able to utilize glucose, xylose, D-mannitol, maltose, lactose and saccharose. No other positive reactions are observed using API 20NE, API 50CH, API Coryne and API ZYM. Growth occurs in the presence of 5% NaCl and 6.5% NaCl. No gas formation is observed in Standard Nutrient I broth. Shows γ-haemolysis on blood agar. The major respiratory quinone is MK-10, followed by MK11 and MK-9. Glucose, mannose, rhamnose and ribose are detected as major whole cell sugars. The peptidoglycan type is a variant of type B2γ with {Gly} [L-DAB/L-Hse] Glu-L-DAB. The polar lipids are one diphosphatidylglycerol, one phosphatidylglycerol, seven glycolipids, one phospholipid and one lipid.

The type strain ZW T2_19^T (= DSM 114294^T = LMG 32700^T) was isolated in Karlsruhe (Germany) from a bulb of an onion landrace (*Allium cepa* var. Rijnsburger) grown in Kleinhohenheim (Germany). The genome size of the type strain is 4.0 Mbp and the DNA G+C content is 71.8 mol%.The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain ZW T2_19^T (= DSM 114294^T = LMG 32700^T) is ON892742. The whole genome shotgun project of strain ZW T2_19^T has been deposited at GenBank/EMBL/DDBJ under the genome accession number JAMRYM000000000.

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

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