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NOTE

Rathayibacter caricis sp. nov. and *Rathayibacter festucae* sp. nov., isolated from the phyllosphere of *Carex* sp. and the leaf gall induced by the nematode *Anguina graminis* on *Festuca rubra* L., respectively

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Two novel species, *Rathayibacter caricis* sp. nov. (type strain VKM Ac-1799^T = UCM Ac-618^T) and *Rathayibacter festucae* sp. nov. (type strain VKM Ac-1390^T = UCM Ac-619^T), are proposed for two coryneform actinomycetes found in the phyllosphere of *Carex* sp. and in the leaf gall induced by the plant-parasitic nematode *Anguina graminis* on *Festuca rubra* L., respectively. The strains of the novel species are typical of the genus *Rathayibacter* in their chemotaxonomic characteristics and fall into the *Rathayibacter* 16S rDNA phylogenetic cluster. They belong to two separate genomic species and differ markedly from current validly described species of *Rathayibacter* at the phenotypic level. The most striking feature differentiating *Rathayibacter caricis* sp. nov. from other species of the genus is the presence of fucose in its cell wall and *Rathayibacter festucae* sp. nov. can be easily recognized among other yellow-pigmented rathayibacters because of its rose-orange-coloured colonies.

Keywords: *Rathayibacter caricis* sp. nov., *Rathayibacter festucae* sp. nov., plant-parasitic nematode, *Anguina*, phyllosphere

The genus *Rathayibacter* was proposed by Zgurskaya *et al.* (1993) for tree species of plant-pathogenic coryneform bacteria, *Rathayibacter tritici*, *Rathayibacter rathayi* and *Rathayibacter iranicus*, previously attributed to the genus *Clavibacter* (Davis *et al.*, 1984). Later, the species *Clavibacter toxicus* (Riley & Ophel, 1992) was reclassified in this genus (Sasaki *et al.*, 1998). Rathayibacters form a coherent cluster within the *Microbacteriaceae* phylogenetic branch (Rainey *et al.*, 1994; Takeuchi & Yokota, 1994; Sasaki *et al.*, 1998) and are characterized by the following chemotaxonomic properties: peptidoglycan of the B type based on 2,4-diaminobutyric acid (L-isomer); major menaquinone of MK-10; phosphatidylglycerol and diphosphatidylglycerol as principal phospholipids; and predominantly saturated anteiso- and iso-methyl branched fatty acids (Zgurskaya *et al.*, 1993; Sasaki *et al.*, 1998). Species differentiation of rathayibacters was traditionally based on physiological characteristics and matched the nature of the plant disease and plant host (Dye & Kemp, 1977). Subsequently, *Rathayibacter* species were shown to differ essentially in their cellular protein patterns (Carlson & Vidaver, 1982), cell-wall sugar composition (Davis *et al.*, 1984; Zgurskaya *et al.*, 1993), enzyme profiles (De Bruyne *et al.*, 1992) and polyamine content (Altenburger *et al.*, 1997), as well as allozyme pattern (Riley *et al.*, 1988).

The main habitats of rathayibacters are considered to be their respective plant hosts, *Triticum aestivum* L., *Dactylis glomerata* L., *Lolium rigidum* Gaud. and some related grasses (Sabet, 1954; Williams, 1964; Dye & Kemp, 1977; Carlson & Vidaver, 1982; Bradbury, 1986; Collins & Bradbury, 1986, 1991; Riley & Ophel, 1992). *Rathayibacter* spp. cause gumming diseases, usually characterized by yellow bacterial slime on seedheads, stems and leaves of the plant host (Sabet, 1954; Gupta & Swarup, 1972; Vidaver, 1982; Carlson

The GenBank accession numbers for the 16S rDNA sequences of *Rathayibacter caricis* sp. nov. VKM Ac-1799^T and *Rathayibacter festucae* sp. nov. VKM Ac-1390^T are AF159364 and AF159365, respectively.

Table 1. Characteristics that differentiate *Rathayibacter caricis* sp. nov., *Rathayibacter festucae* sp. nov. and other species of the genus *Rathayibacter*

Abbreviations: +, positive; −, negative; w, weak or negative, depending on growth phase. Strains: 1, *Rathayibacter caricis* sp. nov. VKM Ac-1799^T; 2, *Rathayibacter festucae* sp. nov. VKM Ac-1390^T; 3, *R. rathayi* VKM Ac-1601^T; 4, *R. tritici* VKM Ac-1603^T; 5, *R. iranicus* VKM Ac-1602^T; 6, *R. toxicus* VKM Ac-1600^T.

Characteristic	1	2	3	4	5	6
Colony colour	Yellow	Rose-orange	Yellow	Yellow	Yellow	Yellow
Observation of visible growth (days)	2	2	2	2	2	4
Cell-wall sugar:*						
Glucose	1·0	1·0	+	+	+	+
Galactose	−	−	+	+	−	−
Mannose	4·5	1·8	+	+	+	+
Rhamnose	7·0	1·3	+	+	+	+
Xylose	0·7	0·3	+	+	−	−
Fucose	1·8	−	−	−	−	−
Oxidase	w	+	w	w	w	w
Methyl red test	+	+	−	−	−	−
Voges–Proskauer	+	+	−	−	−	−
Utilization of:						
Dulcitol	+	+	−	−	−	−
meso-Inositol	+	+	−	−	−	−
Inulin	+	+	−	+	+	−
Melibiose	+	+	−	−	−	−
meso-Erythritol	−	+	−	−	−	−
Salicin	+	+	−	+	+	−
Sorbitol	+	+	−	+	−	−
Hydrolysis of:						
Tween 60	−	+	+	+	+	−
Tween 80	−	+	−	+	+	−
Tolerance to:						
5% NaCl	+	−	−	+	−	−
0·03% Tellurium acetate	−	−	+	+	+	−
Source plant†	*Carex* sp.	*Festuca rubra* L.	*Dactylis glomerata* L.	*Triticum aestivum* L.	*Triticum aestivum* L.	*Lolium rigidum* Gaud.
Nematode associated‡	No data	*Anguina graminis*	*Anguina* sp.§	*Anguina tritici*	*Anguina tritici*	*Anguina funesta*‖

* For strains 1 and 2, molar ratios of the cell-wall sugars are given.

† Data from Dye & Kemp (1977), Carlson & Vidaver (1982), Riley & Ophel (1992) and this study.

‡ Data from Sabet (1954), Gupta & Swarup (1972), Price *et al.* (1979), Riley (1987), Riley & McKay (1990) and this study. *Rathayibacter rathayi* was also demonstrated to be transmitted by *Anguina tritici* into *Triticum* spp. and initiate a disease similar to that caused by *Rathayibacter tritici* (Sabet, 1954; Riley, 1987).

§ Several grasses, including *Dactylis glomerata* L., were previously reported to be hosts of *Anguina agrostis* (Kirjanova & Krall, 1972; Southey, 1973). However, *Anguina* sp. parasitizing *Dactylis glomerata* is phylogenetically distant from *Anguina agrostis sensu stricto* occurring on *Agrostis capillaris* (Powers *et al.*, 2001; S. A. Subbotin, unpublished results).

‖ *Anguina funesta* was considered synonymous with *Anguina agrostis* by some authors (Stynes & Bird, 1980).

& Vidaver, 1982). *Rathayibacter toxicus* (referred to in earlier publications as *Corynebacterium* sp., *Corynebacterium rathayi*, annual raygrass toxicity bacteria or 'ARGT' bacteria) can produce a neurotoxin that can be fatal to grazing animals (Bird, 1981; Vogel *et al.*, 1982; Jago *et al.*, 1983; McKay & Ophel, 1993; Ophel *et al.*, 1993). Rathayibacters use as vectors the highly specialized plant-pathogenic nematodes of the genus *Anguina* (Southey, 1973; Price *et al.*, 1979; Riley, 1987; Southey *et al.*, 1990; Krall, 1991) and can inhabit many galls induced by the nematodes in the various organs of their host plants (Sabet, 1954; Gupta & Swarup, 1972; Stynes & Bird, 1980; Bird, 1981; Evtushenko *et al.*, 1994). Each of the unambiguously identified species of *Rathayibacter* was observed or supposed to be associated with a particular nematode species, *Anguina tritici*, *Anguina funesta* or *Anguina agrostis* (Table 1) (Sabet, 1954; Gupta & Swarup,

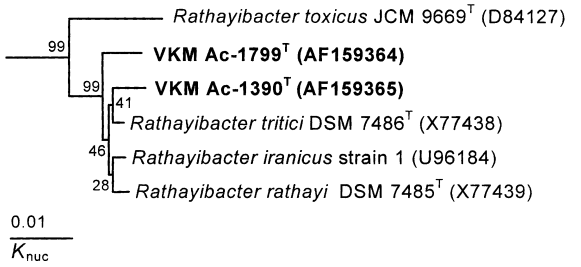

Fig. 1. Phylogenetic tree showing the position of strains VKM Ac-1390^T and VKM Ac-1799^T within the genus *Rathayibacter* based on 16S rDNA analysis. Sequences of the strain *Brevibacterium linens* DSM 20425^T (X77451) served as an outgroup sequence. Other sequences used in the analysis are given in the text. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 1000 bootstrapped trees. Bar, 1 nt substitution per 100 nt.

1972; Price *et al*., 1979; Kirjanova & Krall, 1972; Riley, 1987; Riley & McKay, 1990; Collins & Bradbury, 1991; Riley & Ophel, 1992). However, *Anguina agrostis* was shown to be a heterogeneous species (Southey, 1973), which was supported by molecular analyses (Powers *et al*., 2001; S. A. Subbotin, unpublished results). *Anguina agrostis sensu stricto* is phylogenetically distant from *Anguina* sp. parasitizing *Dactylis glomerata* (Powers *et al*., 2001) and occurs most probably only on *Agrostis capillaris* L. (syn. *Agrostis tenuis* L.) (Riley, 1987; S. A. Subbotin, unpublished results). It should also be noted that *Anguina tritici* was demonstrated to transmit *R. rathayi* into *Triticum* spp. and initiate a disease similar to that caused by *R. tritici* (Sabet, 1954; Riley, 1987).

In the course of studying micro-organisms from plants, two novel strains of rathayibacters were isolated from the phyllosphere of *Carex* sp. and the leaf gall induced by fescue leaf gall nematode *Anguina graminis* on *Festuca rubra* L. In this work, results of their taxonomic study are presented and it is proposed to accommodate these bacteria into two novel species, *Rathayibacter caricis* sp. nov. and *Rathayibacter festucae* sp. nov. Plants of *Festuca rubra* L. infected by the nematode *Anguina graminis* were collected in Moscow region (Russia) in 1991 and maintained at room temperature for 2 years before the study. To isolate bacteria, the gall surface was sterilized with 75% (v/v) ethanol for 1 min and dried; the galls were cut into pieces, added to 2 ml 0·85% (w/v) NaCl solution and milled. The plants of *Carex* sp. (without any visible symptoms of disease) were collected in the Central Chernozem Nature Park (Belgorod Region, Russia) in 1996 and studied immediately. For isolation of bacteria from the phyllosphere, an overhead part of a fresh plant was placed into a flask with saline solution (0·85% NaCl) and shaken for 1 h on a rotary shaker. The above plant gall and phyllosphere suspensions were plated by adding 1 drop into corynebacterium agar (CB agar) (Zgurskaya *et al*., 1993) and incubated for 1 month at room temperature (18–24 °C) in daylight. Bacteria used in this study were isolated after 7 days incubation. The type strains of species of *Rathayibacter* used for the comparative study are listed in Table 1. Morphology and life cycle were observed in cultures grown on CB agar by phase-contrast microscopy. Motility was examined by the hanging-drop method. Physiological features were studied as described previously (Zgurskaya *et al*., 1993; Evtushenko *et al*., 2000). For chemotaxonomic analysis, strains were grown in a liquid CB medium on a rotary shaker at 24 °C for 36 h. Chemotaxonomic characteristics were studied by standard methods as described previously (Evtushenko *et al*., 2000). The G+C content was determined by thermal denaturation and DNA–DNA relatedness was studied by the membrane filter method as reported by Evtushenko *et al*. (2002). The 16S rRNA gene was amplified, sequenced and analysed as given by Evtushenko *et al*. (2000). The sequences of strains VKM Ac-1799^T and VKM Ac-1390^T were compared with those of *Rathayibacter* spp. shown in Fig. 1 and the following sequences of other representatives of the family *Microbacteriaceae* available from GenBank: *Agrococcus jenensis* DSM 9580^T (X92492), *Agromyces cerinus* subsp. *cerinus* DSM 8595^T (X77448), *Agromyces mediolanus* JCM 9633 (D45056), *Agromyces ramosus* DSM 43045^T (X77447), '*Brevibacterium helvolum*' DSM 20419 (X77440), *Clavibacter michiganensis* subsp. *michiganensis* DSM 46364^T (X77435), *Clavibacter michiganensis* subsp. *nebraskensis* LMG 5627^T (U09763), *Cryobacterium psychrophilum* JCM 1463^T (D45058), *Curtobacterium luteum* DSM 20542^T (X77437), *Curtobacterium citreum* DSM 20528^T (X77436), *Microbacterium barkeri* DSM 20145^T (X77446), *Microbacterium imperiale* DSM 20530^T (X77442), *Microbacterium testaceum* DSM 20166^T (X77445), *Leifsonia aquatica* DSM 20146^T (X77450), *Leifsonia xyli* subsp. *cynodontis* MDE1 (M60935) and *Leucobacter komagatae* IFO 15245^T (AB007419).

The results of isolation of bacteria from plant sample suspensions showed that coryneform bacteria with yellow colonies were predominant among micro-organisms isolated from the fescue leaf galls; only a few rose-orange colonies similar to that of strain VKM Ac-1390^T were observed. In contrast, bacteria grown from the phyllosphere of *Carex* sp. revealed much greater diversity of bacterial types and *Pseudomonas*-like bacteria occurred in significant proportions. Among representatives of all the bacterial types isolated from these sources (and other numerous plant samples analysed recently, data not presented), only two strains, VKM Ac-1390^T and VKM Ac-1799^T, were found to belong to the genus *Rathayibacter* and are described in this work.

Yellow-pigmented strain VKM Ac-1799^T from the phyllosphere of *Carex* sp. formed circular, convex, butyrous, opaque colonies of about 1·0 mm in diameter, which were visible after 2–3 days incubation. In young cultures (12–20 h), cells were non-motile, irregular rods (0·5–0·8 × 2·0–2·5 μm) and occurred

singly or in pairs with diphtheroid arrangements. Primary branching was not observed. In 5–7-day-old cultures, the rods were replaced by coccoid or coccobacillus forms, 0·6–1·0 μm in diameter, occurring singly or arranged in pairs, short chains or clumps. A marked life cycle on CB agar and some other media was observed. The rose-orange-pigmented bacterium from the leaf gall of *Festuca rubra* L., strain VKM Ac-1390^T, was generally similar to the isolate from *Carex* sp. phyllosphere in its morphology and the presence of a life cycle, but its cells, which were rods in young culture and cocco-bacillus forms in 5–7-day-old culture, appeared to be less variable in their diameter, which was about 0·7–0·8 μm. Both strains under study were aerobic, catalase-positive and mesophilic, with a thermal optimum for growth of 24–26 °C; no growth was observed at 7 or 37 °C. The oxidase test with tetramethyl-*p*-phenylenediamine was positive in strain VKM Ac-1390^T and weakly positive or negative, depending on the growth phase, in VKM Ac-1799^T. The strains used a wide spectrum of carbon and nitrogen sources for growth (see species description) and differed from each other and from the previously described *Rathayibacter* spp. in these and other phenotypic properties (Table 1). Peptidoglycans of both strains contained glycine, glutamic acid, 2,4-diaminobuthyric acid and alanine in molar ratios of 1·0:1·0:1·93:0·87 (VKM Ac-1799^T) and 1·0:1·03:1·72:0·76 (VKM Ac-1390^T). The amino acid ratio was consistent with peptidoglycan type B2γ (Schleifer & Kandler, 1972). Glucose, mannose, rhamnose and xylose were determined in the cell wall of strain VKM Ac-1390^T in a molar ratio of 1·0:1·8:1·3:0·3, whereas in VKM Ac-1799^T, glucose, mannose, rhamnose, xylose and also fucose were present in a molar ratio of 1·0:4·5:7·1:0·7:1·8. Both strains had MK-10 as predominant menaquinone; menaquinones MK-8, MK-9 and MK-11 were minor components. The diagnostic phospholipids were phosphatidylglycerol and diphosphatidylglycerol; traces of another unidentified phospholipid with a low R_F value were revealed. No mycolic acids were found. The fatty acid profiles of strains VKM Ac-1390^T and VKM Ac-1799^T were typical of *Rathayibacter* and most genera of the family *Microbacteriaceae* and consisted mainly of saturated anteiso- and iso-methyl branched acids as follows (compositions are given for strains VKM Ac-1390^T and VKM Ac-1799^T, respectively): anteiso-15:0 (41 and 43%), anteiso-17:0 (23 and 20%) and iso-16:0 (18 and 21%). Iso-14:0, iso-15:0, iso-17:0 and the non-branched acids were present in variable amounts (up to 3%) or not detected. No 10-methyl branched or ω-cyclohexyl undecanoic acids were identified. The DNA G+C contents of VKM Ac-1390^T and VKM Ac-1799^T were 68·4 and 68·2 mol%, respectively.

To establish the phylogenetic position of the strains, the nearly complete sequence of the 16S rRNA gene, 1496 bases, was determined for strain VKM Ac-1799^T and a sequence of 1270 bases was determined for VKM Ac-1390^T. Their comparison with reference 16S rDNA sequences demonstrated that both isolates fell into the *Rathayibacter* phylogenetic cluster (Fig. 1) and they showed 98·7% 16S rDNA sequence similarity with each other. Both strains were most closely related to *R. tritici*, *R. rathayi* and *R. iranicus*, with 98·8–99·3% 16S rDNA similarities, and exhibited 97·1–97·2% similarity with the type strain of *R. toxicus*. The mean DNA–DNA relatedness between these strains was 36%. VKM Ac-1390^T exhibited 42, 39 and 37% DNA relatedness with the type strains of *R. rathayi*, *R. tritici* and *R. iranicus*, respectively, and VKM Ac-1799^T showed corresponding DNA relatedness of 25, 26 and 22% with these strains. The results indicate that our strains are representatives of two separate genomic species. Thus, on the basis of both phenotypic and molecular genetic findings, it is proposed that strains VKM Ac-1799^T and VKM Ac-1390^T should be classified as two novel species of the genus *Rathayibacter*, *R. caricis* sp. nov. and *R. festucae* sp. nov., respectively. The most striking feature differentiating *R. caricis* sp. nov. from other species of the genus is the presence of fucose in its cell wall and *R. festucae* sp. nov. can be easily recognized among other yellow-pigmented rathayibacters by its rose-orange colonies. Other phenotypic characteristics which can serve to distinguish the novel species at the phenotypic level are listed in Table 1.

It should also be noted that *R. caricis* sp. nov. VKM Ac-1799^T and *R. festucae* sp. nov. VKM Ac-1390^T originate from the plants *Carex* sp. and *Festuca rubra* L., respectively, plants which have not previously been described as hosts for unambiguously identified strains of *Rathayibacter*. Also, neither rathayibacters nor other micro-organisms were observed to be associated with the nematode *Anguina graminis*. The discovery of unidentified coryneform bacteria in the seed galls of fescue (*Festuca rubra* subsp. *commutata* Gaud.) infected by the nematode larvae of *Anguina* sp. (cited as *Anguina agrostis*) and toxic to animals was reported by Galloway (1961). The presence of similar bacteria in the same grass could also be presupposed from earlier publications in 1943–1949 (Bird, 1981) on toxicity to livestock of grass infested with *Anguina* sp. (*Anguina agrostis*). In addition, the orange-pigmented bacteria that are similar morphologically to *R. tritici* (*Corynebacterium tritici*) were found in orchard grass (Williams, 1964). However, there are no available data to show whether the above bacteria belong to *R. festucae* sp. nov., *R. toxicus* or represent other novel species of *Rathayibacter* or other genera.

Visible symptoms of nematode infection in *Carex* sp., from which strain VKM Ac-1799^T was isolated, were not found, but the sedge species are known to be host plants for the nematode *Heteroanguina caricis* (Solovyeva & Krall, 1983). Proceeding from this and relevant knowledge in the biology and habitats of highly specialized plant-parasitic nematodes of the subfamily Anguininae and also the known rathayibacters, one may suppose that *R. caricis* sp. nov. is also associated with a nematode of the subfamily

Anguininae and that this nematode most probably is not *Anguina tritici*, *Anguina funesta*, *Anguina graminis*, *Anguina agrostis* or *Anguina* sp. parasitizing *Dactylis glomerata* L. The difference between *R. caricis* sp. nov. and validly described species of rathayibacters, and also *R. festucae* sp. nov., in its cell-wall and whole-cell sugars (data not presented) might be another basis for the above supposition, since the bacterial polysaccharide capsule has been shown to be involved in bacterial adhesion to the nematode cuticle (Bird, 1985) and the adhesion pattern correlated with the taxonomic groupings of *Anguina* spp. (Riley & McKay, 1990).

There is no unambiguous evidence to show if *R. festucae* sp. nov. and *R. caricis* sp. nov. are plant pathogens *per se* or only associated or could be associated with plant-parasitic nematodes. The plant disease symptoms typical of gumming disease caused by known rathayibacters were not observed and the representatives of the novel species of *Rathayibacter* made up only a small proportion of the microorganisms grown from both plant samples. On the other hand, there are no available data on the discovery of rathayibacters in healthy plants without bacterial or nematode infection. In addition, a little yellow slime on leaves and heads (the main symptom of plant disease) may be unnoticed, e.g. in dry weather or in dried plants (Brown *et al.*, 1989). Finally, most members of the population of the discussed *Rathayibacter* species may be unculturable under the conditions used by us for isolation of plant bacteria. Further studies are required to elucidate the questions pertinent to plant pathogenicity and occurrence of *R. festucae* sp. nov. and *R. caricis* sp. nov., a range of their plant hosts and nematode vectors, their possible toxicity for grazing animals and also their role in the nematode–bacteria complexes parasitizing grasses.

Description of *Rathayibacter caricis* sp. nov.

Rathayibacter caricis (ca.ri.cis. M.L. n. *Carex* sedge; M.L. gen. n. *caricis* of *Carex*, generic name of the plant where the type strain of this species was found).

Colonies on corynebacterium agar are circular, yellow and convex. Cells are non-spore-forming, non-motile, irregular rods. A life cycle may be observed. Obligately aerobic, catalase-positive. Oxidase test with tetramethyl-*p*-phenylenediamine is negative or weakly positive, depending on growth phase. Optimum growth temperature is 24–26 °C; no growth observed at 7 or 37 °C. D-Glucose, D-galactose, adonitol, L-arabinose, cellobiose, dulcitol, D-fructose, *meso*-inositol, inulin, D-lactose, maltose, mannose, mannitol, melibiose, melizitose, raffinose, L-rhamnose, salicin, sorbitol, trehalose, turanose and D-xylose are utilized as carbon sources for growth on salt medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) casitone. Dextran, fucose, lyxose, *meso*-erythritol, ribose and sorbose are not utilized. Asparagine, glycine, leucine, methionine, proline and tyrosine are used as nitrogen sources for growth. Alkaline reaction is positive with fumarate, malate, maleinate and succinate, but negative with acetate, citrate, glutamate, formate, lactate, oxalate, palmetinate, propionate, stearate and tartrate. Tween 40 is hydrolysed, but Tween 60, Tween 80, starch, aesculin, hypoxanthine and xanthine are not. H_2S is produced (weakly). Methyl red and Voges–Proskauer tests are positive. Weak growth with 5 % (w/v) NaCl. Resistant to ampicillin (10 μg ml^{-1}), gentamicin (10 μg ml^{-1}) and rubomicin (10 μg ml^{-1}). Susceptible to karbonicillin, levomicetin, metacycline, rifampicin and streptomycin in the same concentrations and 0·03 % (w/v) potassium tellurite and 0·03 % (w/v) tellurium acetate. DNA G+C content is approximately 68 mol %. Cell-wall sugars consist of predominantly rhamnose and mannose; minor sugars are fucose, glucose and xylose. Other chemotaxonomic characteristics are as given previously (Zgurskaya *et al.*, 1993; Sasaki *et al.*, 1998). The type strain of the species, VKM Ac-1799^T (= UCM Ac-618^T), was isolated from phyllosphere of *Carex* sp. (Central Chernozem Nature Park, Belgorod Region, Russia).

Description of *Rathayibacter festucae* sp. nov.

Rathayibacter festucae (fes′tu.ca.e. M.L. adj. *festucae* from *Festuca*, generic name of fescue, a host plant of this species).

The colonies on corynebacterium agar are circular, convex and orange to rose-orange. The cells are non-spore-forming, non-motile, irregular rods, sometimes curved. Rod–coccoid life cycle may be observed. Obligately aerobic. Catalase- and oxidase-positive. Optimum growth temperature is 24–26 °C; no growth observed at 7 or 37 °C. D-Glucose, D-galactose, adonitol, L-arabinose, cellobiose, *meso*-erythritol, fructose, inositol, inulin, maltose, mannose, mannitol, melibiose, raffinose, L-rhamnose, salicin, sorbitol, trehalose, turanose and D-xylose are used as carbon sources for growth in salt medium supplemented with 0·1 % (w/v) yeast extract and 0·1 % (w/v) casitone. Dextran, lyxose, ribose and sorbose are not utilized. Asparagine, glycine, methionine, proline, tyrosine are used as nitrogen sources for growth. Acetate, citrate, fumarate, gluconate, α-ketoglutarate, malate, malonate, succinate, tartrate are utilized (alkaline reaction), but not formate or propionate. Tween 40, Tween 60 and Tween 80 are hydrolysed, but starch, aesculin, hypoxanthine and xanthine are not. H_2S is produced. Methyl red and Voges–Proskauer tests are positive. No growth with 5 % (w/v) NaCl, 0·03 % (w/v) tellurium acetate and 0·03 % (w/v) potassium tellurite. Resistant to the following antibiotics (10 μg ml^{-1}): ampicillin, gentamicin, oxacillin and rubomicin. Susceptible to doxycycline, levomicetin, metacycline, rifampicin and streptomycin at the same concentration. DNA G+C content is approximately 68 mol %. Cell-wall sugars consist of glucose, mannose

and rhamnose and minor amounts of xylose. Other chemotaxonomic characteristics are as given previously (Zgurskaya *et al*., 1993; Sasaki *et al*., 1998). The type strain of the species, VKM Ac-1390^T (= UCM Ac-619^T), was isolated from the leaf gall induced by the fescue leaf gall nematode *Anguina graminis* on *Festuca rubra* L. (Moscow region, Russia).

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