Rathayibacter gen. nov., Including the Species *Rathayibacter rathayi* comb. nov., *Rathayibacter tritici* comb. nov., *Rathayibacter iranicus* comb. nov., and Six Strains from Annual Grasses

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A new genus, *Rathayibacter*, is proposed to accommodate three species of gram-positive, aerobic, coryneform bacteria previously placed in the genus *Clavibacter* (*Rathayibacter rathayi* comb. nov., *Rathayibacter tritici* comb. nov., and *Rathayibacter iranicus* comb. nov.), as well as six strains that were isolated from annual cereal grasses, may be responsible for ryegrass toxicity, and are very similar to the recently described organism *Clavibacter toxicus* sp. nov. (I. T. Riley and K. M. Ophel, Int. J. Syst. Bacteriol. 42:64–68, 1992). The properties of members of the genus *Rathayibacter* include coryneform morphology, peptidoglycan based on 2,4-diaminobutyric acid (type B2 γ), predominant menaquinones of the MK-10 type, and phosphatidylglycerol and diphosphatidylglycerol as basic polar lipids. The DNA base compositions range from 63 to 72 mol% G+C. The members of the new genus form a phenetic cluster distinct from *Clavibacter* spp. at a level of 71% (simple matching coefficient) and exhibit 7 to 9% DNA-DNA reassociation with strains of *Clavibacter* spp. In contrast to *Clavibacter* spp., most *Rathayibacter* strains are associated with nematodes belonging to the genus *Anguina*. The *Rathayibacter* species differ from species belonging to related genera (e.g., *Clavibacter* and *Agromyces* species) in the following characteristics: menaquinone and whole-cell sugar compositions, results of lysozyme-sodium dodecyl sulfate test (which indicates differences in cell wall composition), ability to utilize a number carbon sources, resistance to bacteriocins of some *Clavibacter* spp., and other characteristics. The *Rathayibacter* species can be differentiated from each other by following characteristics: presence or absence of xylose and galactose in the cell walls, fatty acid composition, ability to assimilate various sources of carbon and nitrogen, hydrolytic activity, tolerance to 5% NaCl and 0.03% potassium tellurite, susceptibility to iranycin, and absence or presence of plasmids of certain sizes. The type species of the genus *Rathayibacter* is *R. rathayi*. The type strains of the species are *R. rathayi* VKM Ac-1601 (= ICMP 2574), *R. iranicus* VKM Ac-1602 (= ICMP 3496), and *R. tritici* VKM Ac-1603 (= ICMP 2626).

Until recently, gram-positive aerobic coryneform bacteria with 2,4-diaminobutyric acid (DAB) in their cell walls were placed in the genus *Corynebacterium* Lehmann and Neumann 1896 (3, 7, 25, 28, 41, 43, 49). However, accumulated evidence (2, 3, 5, 11, 15, 16, 18, 27, 31, 42, 46) has shown that there is great heterogeneity in this genus as currently circumscribed. Among other things reflecting this heterogeneity is the suggestion (7) that a group of plant-pathogenic *Corynebacterium* species should be distinguished.

Davis et al. (13) proposed the genus *Clavibacter* for those coryneform phytopathogenic bacteria that contain DAB as a marker cell wall component. Accordingly, several species and subspecies were transferred to the genus *Clavibacter* as *Clavibacter iranicus*, *Clavibacter michiganensis* subsp. *michiganensis*, *Clavibacter michiganensis* subsp. *insidiosus*, *Clavibacter michiganensis* subsp. *nebraskensis*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Clavibacter michiganensis* subsp. *tessellarius*, *Clavibacter rathayi*, and *Clavibacter tritici*; in addition, a new species with two subspecies, *Clavibacter xyli* subsp. *xyli* and *Clavibacter xyli* subsp. *cynodontis*, was described. Another new species, *Clavibacter toxicus*, has been proposed recently by Riley and Ophel (38) for bacteria responsible for annual ryegrass toxicity. The characteristics that differentiate species in the genus

Clavibacter as currently circumscribed include menaquinone type (MK-9 and/or MK-10), composition of cell wall sugars, G+C content, pigmentation, and selected physiological features (13, 38).

However, at the present time the differences in predominant menaquinone types (together with phylogenetic affinity data and other selected phenotypic characteristics) form the basis for differentiating some coryneform genera (9, 12, 44). The differences in menaquinone composition, as well as morphological and physiological features, are the basis for distinguishing between the related genera *Clavibacter* and *Agromyces* (the members of the genus *Agromyces* also have a peptidoglycan based on DAB) (4, 54). In addition, some genera of coryneform bacteria that are significantly remote phylogenetically may still have no generally acceptable differentiating chemotaxonomic characteristics (10). These organisms can be distinguished phenotypically by fatty acid composition and some cultural and physiological properties.

The chemotaxonomic (7, 11, 31), physiological (16), and genetic differences (39) among *Clavibacter* strains, as well as the data mentioned above and the general outlines of development of bacterial systematics (33, 50) available, suggest that the members of the genus might be divided into two genera. Preliminary DNA-DNA and DNA-rRNA reassociation data (15, 46) do not clearly establish the genus *Clavibacter* as a homogeneous or heterogeneous genus.

In this paper, we report the results of a comparative

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TABLE 1. Strains investigated

Taxon	Strain ^a	Geographic region	Source ^b
<i>Clavibacter iranicus</i>	ICMP 3496 ^T	Iran	<i>Triticum aestivum</i>
<i>Clavibacter rathayi</i>	ICMP 2574 ^T	New Zealand	<i>Dactylis glomerata</i>
	ICMP 2571	United Kingdom	<i>Dactylis glomerata</i>
	ICMP 2572	New Zealand	<i>Dactylis glomerata</i>
	ICMP 2576	New Zealand	<i>Dactylis glomerata</i>
	ICMP 2579	Scotland	<i>Dactylis glomerata</i>
<i>Clavibacter tritici</i>	ICMP 2626 ^T	Egypt	<i>Triticum aestivum</i>
	ICMP 2623	India	<i>Triticum aestivum</i>
	ICMP 2624	Egypt	<i>Triticum aestivum</i>
	ICMP 2625	India	<i>Triticum aestivum</i>
	ICMP 2627	India	<i>Triticum aestivum</i>
	ICMP 2628	Iran	<i>Triticum aestivum</i>
" <i>Corynebacterium</i> sp."	ICMP 6306	Australia	<i>Lolium rigidum</i>
	ICMP 6307 ^c	Australia	<i>Lolium rigidum</i>
	ICMP 6308	Australia	<i>Phalaris minor</i>
	ICMP 6309	Australia	<i>Phalaris minor</i>
	ICMP 6310	Australia	<i>Phalaris paradoxa</i>
	ICMP 6311	Australia	<i>Avena fatua</i>
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	ICMP 2550 ^T	Hungary	<i>Lycopersicon esculentum</i>
<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i>	ICMP 3298 ^T	United States	<i>Zea mays</i>
<i>Clavibacter michiganensis</i> subsp. <i>tessellarius</i>	ICMP 7221 ^T	United States	<i>Triticum aestivum</i>
<i>Agromyces ramosus</i>	ATCC 25173 ^T	Unknown	Soil
<i>Agromyces cerinus</i> subsp. <i>cerinus</i>	VKM Ac-1340 ^T	Russia	Soil
<i>Agromyces fucosus</i> subsp. <i>fucosus</i>	VKM Ac-1345 ^T	Russia	Soil

^a ATCC, American Type Culture Collection, Rockville, Md.; ICMP, International Collection of Microorganisms from Plants (formerly Plant Diseases Division Culture Collection), Auckland, New Zealand.

^b Data from references 7, 24, and 54.

^c Recently described as *Clavibacter toxicus* (38).

taxonomic study of *Clavibacter* spp. strains possessing menaquinones of the MK-10 type and six "*Corynebacterium* sp." strains isolated from annual grasses. On the basis of the data obtained, a new genus, *Rathayibacter*, is proposed to accommodate *Rathayibacter iranicus* comb. nov., *Rathayibacter rathayi* comb. nov., *Rathayibacter tritici* comb. nov., and six *Rathayibacter* sp. strains.

MATERIALS AND METHODS

Bacterial strains. The strain designations and the relevant information concerning strain origins are shown in Table 1. All strains were maintained on modified Prauser medium 79 (35) (10 g of glucose, 5 g of peptone, 2 g of yeast extract, 2 g of Casamino Acids, 6 g of NaCl, 15 g of agar, 1 liter of distilled water [pH 7.4]).

Morphology. Morphological characteristics were studied by phase-contrast microscopy of 1-, 3-, and 7-day-old cultures grown on Prauser medium 79.

Physiology. Utilization of carbohydrates was tested by using modified ISP 9 medium (36) supplemented with 0.1% (wt/vol) yeast extract and 0.1% Casamino Acids. The different carbon sources were added at final concentrations of 1% (wt/vol). Basal medium with no added carbon source and basal medium supplemented with glucose were used for negative and positive controls, respectively. Modified Christensen medium (51) without glucose was used to assay the assimilation of organic acids at pH 6.8; the different organic acids were added at final concentrations 0.5% (wt/vol). Production of acids from carbohydrates was assayed in a basal medium containing 0.1% (wt/vol) $\text{NH}_4\text{H}_2\text{PO}_4$, 0.02% KCl, 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% yeast extract, 0.1% Casamino Acids, 0.004% bromocresol purple, and 1.5% agar. A pronounced change of indicator color was defined as

a positive reaction. Utilization of amino acids as sole sources of nitrogen was tested on a medium containing 1% glucose, 0.1% K_2HPO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05% NaCl, 10 mg of biotin per liter, 1 mg of thiamine per liter, and 1.5% agar. Growth on the basal medium supplemented with methionine was used as a positive control. Susceptibility to growth inhibitors and antibiotics was tested on NBY agar (47) modified as follows: the medium contained 0.8% nutrient broth (Difco), 0.2% Casamino Acids, 0.2% yeast extract, 0.5% NaCl, 0.5% glucose, and 1.5% agar. Tests to determine degradation activities, reduction of nitrate, methyl red and Voges-Proskauer reactions, and hydrolysis of hippurate and other tests were performed by methods described previously (17, 19, 20, 26). All assays were done at 28°C unless indicated otherwise; cells grown on NBY agar at 28°C for 48 h were used as inocula.

Bacteriocin production. The procedures of Gross and Vidaver (21) were used to test bacteriocin production on NBY agar and Prauser medium 79 agar.

Chemotaxonomy. Cell wall peptidoglycan preparations were obtained by using the methods of Schleifer and Kandler (42). The amino acid compositions of peptidoglycans were determined with a model LC 600 amino acid analyzer (Biotronic, Munich, Germany). Sugars in whole-cell hydrolysates (1 M HCl, 105°C, 30 min) were analyzed by thin-layer chromatography (23). Cellular fatty acid methyl ethers were determined as described previously (17). Menaquinones were extracted and purified by the methods of Collins (6), and their compositions were assayed by using a model MAT 8430 mass spectrometer (Finnigan, Bremen, Germany). Phospholipids were isolated and analyzed by thin-layer chromatography as described previously (32).

Lysozyme-SDS test. Cell sensitivity to lysing agents (referred to as the lysozyme-sodium dodecyl sulfate [SDS] test) has been found to be useful in studies of plasmid profiles.

The procedure described by Dobritsa (14), with slight modification (52), was used. Cultures were grown on modified Prauser medium 79 at 28°C for 44 h. A 1-ml portion of a culture suspension was harvested in a 1.5-ml microcentrifuge tube by centrifugation, washed once with 300 µl of a solution containing 10% sucrose in 0.05 M Tris-HCl (pH 8.0), and resuspended in 300 µl of the same solution supplemented with 15 µl of 0.5 M disodium EDTA (pH 8.0) and lysozyme (Sigma) (100 mg/ml in TE buffer containing 0.05 M Tris-HCl and 0.02 M disodium EDTA [pH 8.0]) to a final concentration of 10 mg/ml. The cells were allowed to lyse by incubating the suspension in a water bath at 37°C for 20 min. Then, 350 µl of lysing solution (3.8% SDS, 0.19 N NaOH, 0.05 M Tris, 15% sucrose) was added. The lysates were heated at 60°C for 30 min in a water bath. Clearing of lysates was considered a positive reaction.

DNA base composition and DNA-DNA hybridization. DNA was prepared as described previously (17). The G+C content of DNA was determined from its thermal denaturation midpoint (29) in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) by using a Beckman model DU-8B spectrophotometer equipped with a thermoprogrammer. DNA base composition was calculated by using the equation of Owen and Pitcher (34). DNA from *Micrococcus luteus* VKM B-1314^T (T = type strain), which contains 72 mol% G+C (29), was used as a reference. The extents of DNA reassociation between strains were determined by the method of Meyer and Schleifer (30) and Stackebrandt et al. (45), modified as described previously (17).

RESULTS AND DISCUSSION

All *Clavibacter iranica*, *Clavibacter rathayi*, and *Clavibacter tritici* strains and six "*Corynebacterium* sp." strains isolated from *Lolium rigidum*, *Avena fatua*, *Phalaris minor*, and *Phalaris paradoxa* (Table 1) were similar in their basic chemotaxonomic features, including uncommon amino acid composition of purified peptidoglycans (alanine, glycine, glutamic acid, and DAB in a molar ratio of 1:1:1:2), basic phospholipids (phosphatidylglycerol and diphosphatidylglycerol), predominant unsaturated menaquinones with 10 isoprene units (MK-10), and major amounts of iso and anteiso methyl branched-chain fatty acids (Table 2). These characteristics were similar for the type strains of *Clavibacter iranica*, *Clavibacter rathayi*, and *Clavibacter tritici* (2, 5, 7, 11, 18, 31, 42), and these strains differed from strains of *Clavibacter michiganensis* (*Clavibacter michiganensis* subsp. *michiganensis*, *Clavibacter michiganensis* subsp. *insidiosus*, *Clavibacter michiganensis* subsp. *nebraskensis*, *Clavibacter michiganensis* subsp. *tesselarius*, and *Clavibacter michiganensis* subsp. *sepedonicus*) primarily in the type of menaquinones (MK-10 and MK-9, respectively).

The *Clavibacter iranica*, *Clavibacter rathayi*, and *Clavibacter tritici* strains and six "*Corynebacterium* sp." strains also differed from the *Clavibacter michiganensis* subspecies strains in the following characteristics: lysozyme-SDS test (which probably indicates differences in cell wall composition); ability to utilize inositol, melibiose, L-rhamnose, and tagatose (Table 3); ability to produce bacteriocins; and resistance to bacteriocins of some *Clavibacter* spp. (52). In contrast to *Clavibacter michiganensis* strains, most of the strains with menaquinones of the MK-10 type contain xylose but not fucose in their cell walls (Tables 2 and 3).

According to the results of a numerical analysis of 85 strains of *Clavibacter* spp. and related species in which 126

TABLE 2. Differentiating characteristics of *Rathayibacter* species^a

Characteristic	<i>R. rathayi</i> (n = 5) ^b	<i>R. tritici</i> (n = 6)	<i>R. iranica</i> (n = 1)	<i>Rathayibacter</i> sp. (n = 6)
Cell wall sugars				
Galactose	(+) ^c	(+)	+	—
Xylose	+	+	—	—
Fatty acid composition (%)				
iso-15:0	1.8–4.7	0.0–4.3	0.9	0.0–2.3
anteiso-15:0	39.2–73.5	28.7–45.6	42.1	32.7–46.8
iso-16:0	7.6–15.7	15.5–36.1	21.1	10.2–20.4
16:0	0.0–2.7	3.0–5.2	3.3	0.9–1.8
iso-17:0	0.0–3.5	0.0–0.0	1.1	0.4–1.7
anteiso-17:0	10.0–33.8	18.4–38.0	28.0	39.8–46.7
17:0	0.0–0.07	0.0–2.5	0.4	0.9–3.3
Utilization of:				
Xylose	+	+	+	—
L-Arabinose	+	+	+	—
Lactose	+	+	+	—
Mannitol	+	+	+	—
Sorbitol	(+)	+	+	—
Inulin	—	+	—	—
Citrate	+	+	+	—
Tartrate	+	+	+	—
Sebacinate	(+)	+	—	—
Acid production from:				
Glucose	—	+	—	—
Galactose	—	(+)	+	—
Fructose	(+)	+	—	—
Mannose	—	+	+	—
Maltose	—	+	—	—
Xylose	+	+	+	—
Sucrose	—	+	—	—
Amino acid utilization				
DL-Valine	+	—	—	—
Glutamic acid	+	+	+	—
DL-Ornithine	+	+	+	—
Hydrolysis of	+	+	+	—
Tween 21,				
Tween 40,				
and Tween 85				
Tolerance to:				
5% NaCl	—	+	—	—
0.030% potassium tellurite	(+)	+	+	—
Voges-Proskauer test	+	—	—	—
Methyl red test	+	+	—	—
Susceptibility to iranicin ^d	+	—	—	+

^a See Table 1 for information on the strains used. In addition, *R. rathayi* contained 37- and 83-kb plasmids and *R. tritici* contained a 50-kb plasmid. We found no plasmids in *R. iranica* or the *Rathayibacter* sp. strains.

^b n is the number of strains tested.

^c +, positive reaction; —, negative reaction; (+), reaction variable among strains.

^d Iranicin is the bacteriocin of *R. iranica* (21).

unit characters were used (53), all strains containing type MK-10 menaquinones as the predominant menaquinones (*Clavibacter iranica*, *Clavibacter rathayi*, and *Clavibacter tritici* strains and six strains isolated from annual grasses) form a clear cluster distinct from the cluster of *Clavibacter*

TABLE 3. Some diagnostic and differentiating characteristics of the genera *Clavibacter*, *Rathayibacter*, and *Agromyces*

Characteristic	<i>Clavibacter</i>	<i>Rathayibacter</i>	<i>Agromyces</i> ^a
Peptidoglycan based on DAB (type B2 γ)	+ ^b	+	+
Major menaquinone	MK-9	MK-10	MK-12
Cell wall sugars			
Glucose	+	+	(+)
Galactose	+	(+)	+
Mannose	+	+	(+)
Rhamnose	+	+	+
Xylose	—	(+)	(+)
Fucose	(+)	—	(+)
Tyvelose	—	—	(+)
Mycelium	—	—	+
Utilization of:			
Inositol	+	—	(+)
Melibiose	(+)	—	(+)
L-Rhamnose	(+)	—	+
Tagatose	(+)	—	—
Susceptibility to iranin ^c	+	(+)	—
Lysozyme-SDS test	+	—	+
Usual sources of isolation	Plants or plant substrates	Plants	Soil

^a Data from reference 54.^b +, positive reaction; —, negative reaction; (+), reaction variable among strains.^c Iranicin is the bacteriocin of *R. iranicus* (21).

michiganensis subspecies at a level of 71% (simple matching coefficient).

The heterogeneity of the original genus *Clavibacter* is also supported by our DNA-DNA hybridization data (Table 4). DNA relatedness values of 56 and 60% were observed between *Clavibacter michiganensis* subsp. *michiganensis* ICMP 2550^T and two other strains with type MK-9 menaquinones (*Clavibacter michiganensis* subsp. *nebraskensis* ICMP 3298^T and *Clavibacter michiganensis* subsp. *tessellarius* ICMP 7221^T). *Clavibacter iranicus* ICMP 3496^T and *Clavibacter tritici* ICMP 2626^T gave DNA relatedness values

TABLE 4. Levels of DNA relatedness among *Clavibacter*, *Rathayibacter*, and *Agromyces* strains

Strain	% Relatedness to [³ H]DNA from:		
	Strain ICMP 2550 ^T	Strain ICMP 2574 ^T	Strain VKM Ac-1340 ^T
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> ICMP 2550 ^T	100	7	10
<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> ICMP 3298 ^T	60		
<i>Clavibacter michiganensis</i> subsp. <i>tessellarius</i> ICMP 7221 ^T	56		
<i>R. rathayi</i> ICMP 2574 ^T	9	100	12
<i>R. tritici</i> ICMP 2626 ^T	8	43	
<i>R. iranicus</i> ICMP 3496 ^T	8	16	
<i>Agromyces ramosus</i> ATCC 25173 ^T	7	5	12
<i>Agromyces cerinus</i> subsp. <i>cerinus</i> VKM Ac-1340 ^T	10	7	100
<i>Agromyces fucosus</i> subsp. <i>fucosus</i> VKM Ac-1345 ^T			40

of 16 and 43% with *Clavibacter rathayi* ICMP 2574^T, whereas the levels of DNA relatedness between the type strain of *Clavibacter michiganensis* subsp. *michiganensis* and the type strains of *Clavibacter iranicus*, *Clavibacter rathayi*, and *Clavibacter tritici* were 7 to 9%. The levels of DNA relatedness between representatives of the genera *Clavibacter* and *Agromyces* have been found to be in approximately the same range (5 to 10%) (54). These data indicate that the strains of *Clavibacter* spp. which we studied can be separated on the basis of DNA relatedness values into two distinct groups rather than at the species level.

Also, in an independent study, Riley et al. (39) showed that strains of *Clavibacter* spp. may be divided into at least two groups, one centered around *Clavibacter michiganensis* and its subspecies and the other accommodating *Clavibacter rathayi*, *Clavibacter tritici*, and *Clavibacter iranicus*, on the basis of their allelic profiles, which reflect possible genetic relationships among organisms.

The results of immunodiffusion and enzyme-linked immunosorbent assays are difficult to interpret taxonomically as a rule (8). Nevertheless, serological data of this kind indicate that *Clavibacter rathayi*, *Clavibacter tritici*, and *Clavibacter iranicus* are serologically distinct from the other plant-pathogenic corynebacteria, including *Clavibacter michiganensis* (27, 37).

Moreover, in contrast to *Clavibacter michiganensis* and *Clavibacter xyli*, *Clavibacter tritici*, *Clavibacter iranicus*, "*Corynebacterium* sp." strains isolated from *L. rigidum*, and probably *Clavibacter rathayi* are associated with nematodes belonging to the genus *Anguina*, require these animals as vectors (1, 8, 20, 38), and exhibit specific adhesion to *Anguina* species (1). In addition, *Clavibacter rathayi*, *Clavibacter tritici*, *Clavibacter iranicus*, and "*Corynebacterium* sp." strains were isolated only from cereal plants (Table 1) (7, 24, 40, 48).

Thus, the phenotypic and genetic differences, as well as the peculiarities of ecology, of these *Clavibacter* spp. suggest that the coryneform bacteria with type B2 γ peptidoglycan can be separated into two taxa which can be considered separate genera. On the basis of the considerations discussed above, we propose that a new genus, *Rathayibacter*, should be established for three species of phytopathogenic bacteria previously placed in the genus *Clavibacter* (*Rathayibacter rathayi* comb. nov., *Rathayibacter tritici* comb. nov. and *Rathayibacter iranicus* comb. nov.), as well as for *Rathayibacter* sp. strains isolated from *A. fatua*, *L. rigidum*, *P. minor*, and *P. paradoxa* (these strains may be responsible for annual ryegrass toxicity and may be members of the recently described taxon *Clavibacter toxicus* sp. nov. [38]).

The type strains of the species are *R. rathayi* VKM Ac-1601 (= ICMP 2574), *R. iranicus* VKM Ac-1602 (= ICMP 3496), and *R. tritici* VKM Ac-1603 (= ICMP 2626).

The strains of *Rathayibacter* species were differentiated from each other (Table 2) by the following characteristics: presence of xylose and galactose in the cell walls, fatty acid composition, ability to assimilate various sources of carbon and nitrogen, hydrolytic activity, tolerance to 5% NaCl and 0.03% potassium tellurite, susceptibility to iranin, absence or presence of plasmids of certain sizes (52), and specificity for several hosts (Table 1).

The strains referred to above as *Rathayibacter* sp. strains are similar to *R. iranicus* in cell wall sugar composition (glucose, mannose, and rhamnose) (Table 2) but are clearly differentiated from *R. iranicus*, *R. rathayi*, and *R. tritici* by their morphology (shorter rods [0.4 to 0.6 by 0.5 to 1.6 μ m]), nutrient requirements, time needed to reach the stationary

growth phase (4 and 2 days, respectively), physiological properties, absence of plasmids (52), host specificity (Table 1), and the predominant symptoms of diseases (8, 38, 40, 48). A phenotypic similarity analysis (53) showed that *R. rathayi*, *R. tritici*, *R. iranicus*, and the *Rathayibacter* sp. strains isolated from annual grasses form four phena at approximately the same level inside the *Rathayibacter* cluster. Taken collectively, these properties leave no doubt that the *Rathayibacter* sp. strains are members of a separate species.

The data described above indicate the need to formally name the *Rathayibacter* sp. strains isolated from annual grasses. In this paper we do not present a formal proposal because these strains probably are members of the species recently described by Riley and Ophel (38) as *Clavibacter toxicus*, at least judging by the biochemical and physiological criteria used in our work. In addition, some *Rathayibacter* sp. strains were isolated from *L. rigidum*, the same source as all of the *Clavibacter toxicus* strains (38). In fact, one of the strains (strain ICMP 6307) classified as belonging to *Clavibacter toxicus* (38) was included in our study and was recovered in a close cluster (simple matching coefficient, 95%), to which the name *Rathayibacter* sp. was applied. However, we have not had the opportunity to perform side-by-side experimental comparisons with the type strain of *Clavibacter toxicus* (strain ICMP 9525). Perhaps after such a study (by us or other workers) *Clavibacter toxicus* may be transferred to the genus *Rathayibacter* as *Rathayibacter toxicus* comb. nov. For differentiating purposes both the criteria described in our study and those used by Riley and Ophel (38) can be employed.

We feel that our proposal to reclassify some members of the genus *Clavibacter* and establish the new genus *Rathayibacter* for a group of coryneform bacteria with an extensive record of taxonomic and ecological studies might not meet with widespread approval, at least initially. On the other hand, we visualize our effort as a modest step on the important road toward reconciliation of approaches, as broadly outlined previously (33, 50). Our results and suggestions do not seem to contradict the interests of either a coherent taxonomic picture or a practically manageable identification scheme for a group of bacteria with type B2_γ peptidoglycan in their cell walls.

Future studies on the taxonomic structure of bacteria which contain DAB in their cell walls not only might benefit from the data accumulating for representatives of the three genera mentioned above (*Clavibacter*, *Rathayibacter*, and *Agromyces*), but also might take into account information reported previously (7, 11, 18, 31, 39, 48) for such bacteria as "*Corynebacterium aquaticum*," "*Corynebacterium mediolanum*," "*Flavobacterium dehydrogenans*," "*Corynebacterium agropyri*," "*Corynebacterium okanaganae*," "*Brevibacterium helvolum*," and "*Corynebacterium bovis*" ATCC 13722.

Description of *Rathayibacter* gen. nov. *Rathayibacter* (Ra. thay' i. bac. ter. M.L. gen. n. *rathayi*, of Rathay, referring to E. Rathay, the Australian plant pathologist who first isolated members of the genus; L. masc. n. *bacter*, rod; N. L. n. *Rathayibacter*, rod isolated by Rathay). Irregular, pleomorphic, short rods (width, 0.4 to 0.8 μ m; length, 0.5 to 1.8 μ m). V-forms and sometimes short cell chains are formed; a clear rod-coccus cycle is not observed. Nonmotile. Nonsporulating. Gram positive and not acid fast. Obligately aerobic. Chemoorganotrophic, having an oxidative type of catabolism. Catalase positive. Nutritionally exacting. Mesophilic; optimal growth occurs at temperatures between 24 and 28°C.

Cell wall peptidoglycan contains alanine, glutamic acid, glycine, and DAB (1:1:1:2). Cell wall sugars include glucose, mannose, and rhamnose. Xylose and galactose also occur in the cell walls of some strains. Menaquinones are of the MK-10 type. Mycolic acids are absent. Among the fatty acids, branching saturated fatty acids of the anteiso type (anteiso-15:0 and anteiso-17:0) predominate. Diagnostic phospholipids are represented by phosphatidylglycerol, diphosphatidylglycerol, and unknown glycosyldiacylglycerols; several strains contain lyso-phosphatidylglycerol. The G+C contents of DNAs range from 63 to 72 mol%.

Most strains utilize cellobiose, fructose, galactose, glucose, glycerol, maltose, mannose, melezitose, methyl- α -D-arabinopyranoside, raffinose, salicin, sucrose, and trehalose as sole carbon sources but do not utilize adonitol, dextrin, dulcitol, erythritol, inositol, lyxose, mannitol, melibiose, L-rhamnose, tagatose, or sorbitol. No acids are formed from dulcitol, inositol, lactose, mannitol, melibiose, L-rhamnose, or sorbitol. Acid production from other sugars is variable.

Acetate, formate, fumarate, gluconate, glutarate, α -ketoglutarate, lactate, malate, propionate, succinate, and valerate are utilized. Phenylalanine and methionine are assimilated, but most amino acids are not used as sole sources of nitrogen.

Casein, elastin, guanine, hypoxanthine, testosterone, Tween 60, Tween 65, Tween 80, starch, urea, and xanthine are not hydrolyzed or decomposed. Nitrate is not reduced to nitrite. Hydrolysis of hippurate is negative. Methyl red reduction and Voges-Proskauer tests are variable. Growth occurs at temperatures between 16 and 35°C, but not at 10 or 37°C. Susceptible to 7% NaCl and 0.02% potassium tellurite but tolerates 0.01% potassium tellurite.

Some strains produce bacteriocins. Most strains contain plasmids.

Isolated mainly from cereal crops and annual cereal grasses. The type species of the genus is *Rathayibacter rathayi*.

Characteristics of the *Rathayibacter* sp. strains. The *Rathayibacter* sp. strains isolated from annual grasses (see above) had the characteristics described below. Irregular, pleomorphic, short rods (width, 0.4 to 0.6 μ m; length, 0.5 to 1.6 μ m). V-forms and sometimes short cell chains are formed; a clear rod-coccus cycle is not observed. Nonmotile. Nonsporulating. Gram positive and not acid fast. Obligately aerobic. Chemoorganotrophic, having an oxidative type of catabolism. Catalase positive. Nutritionally exacting. Mesophilic; optimal growth occurs at temperatures between 24 and 28°C.

Cell wall sugars include glucose, mannose, and rhamnose.

Most strains utilize cellobiose, fructose, galactose, glucose, glycerol, maltose, mannose, melezitose, methyl-arabinopyranoside, raffinose, salicin, sucrose, and trehalose as sole carbon sources but do not use adonitol, L-arabinose, dextrin, dulcitol, erythritol, inositol, lactose, D-xylose, mannitol, melibiose, L-rhamnose, tagatose, and sorbitol. Acid is produced from galactose. No acids are formed from dulcitol, fructose, glucose, inositol, lactose, maltose, mannitol, melibiose, L-rhamnose, sorbitol, sucrose, and D-xylose. Acetate, formate, fumarate, gluconate, glutarate, α -ketoglutarate, lactate, malate, propionate, succinate, and valerate are utilized. An alkaline reaction is not observed with citrate, malonate, sebacinate, or tartrate. Phenylalanine, glutamic acid, ornithine, and methionine are assimilated, but most amino acids are not used as sole nitrogen sources.

Casein, elastin, guanine, hypoxanthine, testosterone, Tween 21, Tween 40, Tween 60, Tween 80, Tween 85,

starch, urea, and xanthine are not hydrolyzed or decomposed. Nitrate is not reduced to nitrite. Hydrolysis of hippurate is negative. Methyl red reduction and Voges-Proskauer tests are negative. Growth occurs at temperatures between 20 and 35°C but not at 14 or 37°C. Susceptible to 5% NaCl and 0.02% potassium tellurite but tolerates 0.01% potassium tellurite.

Susceptible to bacteriocin produced by *R. iranicus* ICMP 3496^T. Plasmids are not observed. The G+C content of the DNA is 63 mol%.

Isolated mainly from annual cereal grasses.

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