Rathayibacter toxicus, Other *Rathayibacter* Species Inducing Bacterial Head Blight of Grasses, and the Potential for Livestock Poisonings

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ABSTRACT

Rathayibacter toxicus, a Select Agent in the United States, is one of six recognized species in the genus *Rathayibacter* and the best known due to its association with annual ryegrass toxicity, which occurs only in parts of Australia. The *Rathayibacter* species are unusual among phytopathogenic bacteria in that they are transmitted by anguinid seed gall nematodes and produce extracellular polysaccharides in infected plants resulting in bacteriosis diseases with common names such as yellow slime and bacterial head blight. *R. toxicus* is distinguished from the other species by producing corynetoxins in infected plants; toxin production is associated with infection by a bacteriophage. These toxins cause grazing animals feeding on infected plants to develop convulsions and abnormal gate, which is referred to as “staggers,” and often results in death of affected animals. *R. toxicus* is the only recognized *Rathayibacter* species to produce toxin, although reports of livestock deaths in the United States suggest a closely related toxigenic species may be present. A closely related but undescribed species, *Rathayibacter* sp. EV, originally isolated from *Ehrharta villosa* var. *villosa* in South Africa, is suspected of producing toxin. Many of the diseases caused by *Rathayibacter* species occur in arid areas and the extracellular polysaccharide they produce is believed to aid in their survival between crops. For example, *R. “agropyri”* was isolated from infected plant material after being stored for 50 years in a herbarium. Similarly, the anguinid vectors associated with these bacteria form seed galls in infected plants and are capable of surviving for very long periods of time under dry conditions. The addition of *R. toxicus* to the list of Select Agents has raised concern over its potential introduction and a realization that current diagnostic methods are inadequate to distinguish among *Rathayibacter* species. In addition, little is known about the *Rathayibacter* species and their seed gall nematode vectors present in the United States.

Additional keywords: bacteriology, ecology and epidemiology, etiology, nematology.

Rathayibacter toxicus (Riley and Ophel 1992) Sasaki et al. 1998 was listed as a Plant Pathogen Select Agent under 7 CFR 331 by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service in 2008 (Murray et al. 2015). Relatively few plant pathologists would have known of this bacterial plant pathogen prior to its listing as a Select Agent, and fewer still would know that there are five other described species of *Rathayibacter* that cause similar diseases and have similar life cycles in other grass hosts. What is it about this Gram-positive bacterium that makes it such a threat to be considered a Select Agent? *R. toxicus* is transmitted by seed gall nematodes (*Anguina* sp.), some of which

are quarantine pests, to nearby host plants where it causes a bacteriosis disease of leaves and floral structures of several different grass species, but primarily annual ryegrass (*Lolium rigidum* Gaud.). During colonization, *R. toxicus* produces corynetoxins that can result in fatal poisoning of animals that graze on diseased plants, a syndrome now referred to as annual ryegrass toxicity (ARGT). To further complicate the relationship, Bird et al. (1980) and Stynes and Bird (1983) postulated the involvement of a bacteriophage in toxin production. In Australia, this cross-domain pathogen has caused plant disease on over 10 million hectares with losses due to ARGT in 2010 estimated at \$37 million USD (Carslake 2006; Kessell 2010). The potential for damage to the U.S. livestock industry resulted in listing of *R. toxicus* as a Select Agent in 2008 and relisting in 2012. The National Plant Disease Recovery System (NPDRS) subsequently developed a recovery plan for this pathogen (Murray et al. 2015). None of the other five described *Rathayibacter* species have been shown to produce toxins in planta, although reports exist of livestock poisonings in the United States and

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elsewhere after animals grazed on pastures where seed gall nematodes were present (Cunningham and Hartley 1959; Galey et al. 1997; Galloway 1961; Haag 1945; Jensen 1961; Kurochkina and Chizhov 1980; Shaw and Muth 1949).

This review will examine the literature relevant to the history and biology of *Rathayibacter* broadly because they represent a fascinating group of cross-domain pathogens that frequently occur in arid areas (Fig. 1A). As such, both the pathogens and their vectors have developed specialized mechanisms for anhydrobiotic survival, namely production of extracellular polysaccharides (EPS) (Fig. 1B and C) and the formation of galls (Fig. 2A and B), in many of these diseases. Readers primarily interested in *R. toxicus* are directed to the reviews by McKay and Ophel (1993), Riley et al. (2014), and the NPDRS Recovery Plan (Murray et al. 2015), which discuss the relevant literature and provide recommendations for control and surveillance.

ANNUAL RYEGRASS TOXICITY

In 1956, Fisher (1977) received samples of annual ryegrass (*L. rigidum*) from veterinary officers with the South Australia Department of Agriculture that were taken from a pasture where sheep had died after feeding on the grass. Fisher found seed gall nematodes present in the sample and, while visiting the field 2 years later, observed symptoms of bacterial head blight, which he attributed to a *Corynebacterium* species. Nematode seed galls picked from the sample and fed to laboratory mice resulted in the death of one with symptoms similar to those of the dead sheep. By 1967, increases in the numbers of sheep dying annually and affected properties in South Australia were great enough that funds were obtained to begin new research on the problem at the Waite Agricultural Institute (Fisher 1977).

McIntosh et al. (1967) were the first to conclude that a toxin was present where sheep were dying after feeding on infested pastures. They surveyed nine farms where staggers occurred and sampled grass from four of them. Samples were identified to plant species, evaluated for presence of *Anguina agrostis* (Steinbuch 1999) Filipjev 1936, separated into seed and stalk fractions for guinea pig feeding trials, and bulk samples were collected from one property

for sheep feeding trials. Postmortem examinations also were conducted on animals that died of staggers from farms and feeding trials. They found that all pastures contained Wimmera ryegrass (*L. rigidum*) and had generally high nematode infestations. One such pasture with 21% infected seed had the greatest sheep mortality. Experimentally, sheep that grazed on one of the infested pastures developed symptoms typical of staggers and died, as did guinea pigs fed bacteria-infected seed. All had postmortem symptoms including uncoordinated movement and tetanic spasms that were consistent with those of animals that died of staggers described previously. McIntosh et al. (1967) could not conclusively demonstrate whether the toxicity was due to the nematode or the bacterium, but concluded that both were associated with ryegrass staggers. Lanigan et al. (1976) followed-up this research with animal feeding studies and concluded that the bacterial galls and not the nematode galls were the source of toxicity.

Gwynn and Hadow (1971) expanded the reports of toxicity to sheep grazing on Wimmera ryegrass to include pastures in Western Australia. They noted that the disease had occurred in four of the six previous years in one particular pasture. Berry and Wise (1975) coined the term Wimmera rye grass toxicity, or WRGT, for the syndrome in animals associated with grazing pastures infested with seed gall nematodes and bacteria and noted that there were 58 outbreaks on 26 farms with over 3,300 dead sheep and 46 cattle. Up to 60% of the ryegrass sampled from the 26 farms was infected with both *Anguina* sp. and *Corynebacterium* sp. (now *Rathayibacter*), neither of which were identified to species.

Bryden et al. (1991) described the occurrence of a syndrome known as flood plain staggers (FPS) in cattle and sheep in New South Wales, Australia. Samples of blown grass (*Agrostis avenacea* C.C. Gmelin) were collected and used in feeding studies that resulted in animals developing symptoms similar to ARG. Like ARG, a nematode and *Clavibacter* sp. (now *Rathayibacter*) were found in seed heads, and a corynetoxin was extracted from the bacterial galls (Cockrum and Edgar 1985). Davis et al. (1995) later reported that 1,722 cattle, 2,466 sheep, and 11 horses on 31 different properties died as a result of FPS through April 1991. During the same year, Finnie (1991) described a corynetoxin poisoning of sheep in South Australia that was associated with annual beard grass

FIGURE 1

Typical habitat and symptoms of bacterial head blight of wheat grass (*Agropyron* sp.) collected in Idaho and Montana, and colony characteristics of the pathogen. **A**, Overview of roadside collection site with box showing the collection area, **B**, infected wheat grass head, **C**, close-up showing droplets of dried ooze (extracellular polysaccharide) on infected wheat grass head, and **D**, yellow-pigmented colonies of *Rathayibacter* "agropyri" growing on nutrient broth yeast extract agar.

(*Polypogon monspeliensis* (L.) Desf.) and referred to it as Stewart's range syndrome (SRS) (McKay et al. 1993). According to Finnie (1991), the syndrome was indistinguishable from ARG and had been known for about 20 years; *Anguina* sp. nematodes and corynetoxins were confirmed present in the sample from South Australia. McKay et al. (1993) conclusively demonstrated that *Clavibacter toxicus* was present in both *Agrostis avenacea* and *P. monspeliensis* and thus responsible for both FPS and SRS; however, they also demonstrated that the nematode present in these grasses was a new species and not the same one present in annual ryegrass.

Schneider (1981) noted the occurrence of ARG in South Africa in two different districts. In one, Caledon, sheep grazed on a pasture composed of oats, ryegrass and vetch or fed hay harvested from the pasture developed symptoms consistent with ARG and died. In the other, Bredasdorp, cattle deaths occurred on three different farms; in all cases, cattle had grazed on mixed pastures containing Italian (*L. multiflorum* Lam.) and/or annual ryegrass, among other plant species. Examination of hay or plant samples from each of the four pastures revealed the presence of seed gall nematodes identified as *Anguina agrostis*, and a pale yellow, Gram-positive bacterium identified as *Corynebacterium* sp. was isolated. Feeding trials with hay collected from some of the pastures and separated by plant species confirmed the toxicity was associated with the ryegrass and not the other species present.

Grewar et al. (2009) described an outbreak of ARG in horses in the Western Cape Province of South Africa, near Ceres and within 10 km of one of the cattle outbreaks previously reported by Schneider (1981). In this case, horses were fed oat hay contaminated with annual ryegrass; morbidity was limited to two pastures. The

diagnosis was based on symptomatology of affected animals and the presence of bacterial galls in hay fed to the horses.

Nogawa et al. (1997) reported an outbreak of annual ryegrass intoxication in cattle and sheep in Japan. In this case, animals from farms were fed oat hay contaminated with annual ryegrass imported from Australia. ARG was confirmed after detection of corynetoxins in seed from the hay and feeding studies where cattle developed symptoms consistent with the syndrome.

OTHER ANIMAL POISONINGS

Several reports have appeared describing poisonings of grazing animals outside Australia with similarities to ARG, but the presence of a toxigenic bacterium has not been confirmed in any of those cases. In 1941, Mullins (1941) described a disease of sheep, cattle, and horses in New Zealand known as ryegrass staggers because it was found mostly in animals that had grazed on pastures containing predominately ryegrass. Cunningham and Hartley (1959) documented three cases of staggers that occurred in 1957. They were unable to determine the cause of the disease, which occurred during autumn months, but believed it was associated with a toxin of unknown origin in the perennial ryegrass (*Lolium perenne* L.). No subsequent reports of annual ryegrass toxicity-like incidents are found from New Zealand.

Several reports of animal poisonings in the United States with similarities to ARG have appeared. Haag (1945) reported mortality of sheep in Oregon that had consumed screenings of Chewings fescue (*Festuca rubra* subsp. *commutata* Gaud.). He obtained and fed screenings from the same lot to rats and showed that the material

FIGURE 2

A, Yellowish bacterial galls and **B**, dark nematode galls in an inflorescence of *Sporobolus cryptandrus* (Torr.) A. Gray with bacterial head blight collected in Idaho.

was highly toxic. Symptoms included paralysis of rear quarters with swelling and discoloration of rear legs and extensive tissue hemorrhages. Haag (1945) tested several other lots of screenings, confirmed their toxicity to rats, and concluded there was a strong causal relationship between toxicity and *Anguina agrostis* seed gall nematode infestation.

Shaw and Muth (1949) described several cases of cattle and sheep poisonings in Oregon after feeding on various plants including perennial rye (*L. perenne*), pea vine silage, and Chewings fescue screenings. Animals fed on the latter exhibited nervous system symptoms including falling, trembling, and incoordination. Feeding studies of sheep with the fescue screenings resulted in symptomatology and mortality similar to animals that fed on infested pastures. *Anguina agrostis* was present in up to 20% of the seed screenings. The cause of mortality was not confirmed, but presence of the nematode had the strongest association.

Jensen (1961) reported the occurrence and distribution of the grass seed nematode (*Anguina agrostis*) infecting several bentgrass species, Chewings fescue, and orchard grass in Oregon. He also mentioned the occurrence of livestock poisonings in animals fed Chewings fescue screenings containing nematode galls, and attributed it to the combined relationship between the nematode, a bacterium, and the host plant. However, there was no mention of isolation or characterization of the bacterium.

Galloway (1961) described three cases of livestock poisoning associated with feeding Chewings fescue seed screenings from a single farm in Oregon. Toxicity was associated with the presence of heavy infestation of *Anguina* sp. seed gall nematode. Two types of galls were noted in the hay; a brownish one that contained nematode larvae and another that contained a "yellow cheesy material" from which a Gram-positive bacterium resembling *Corynebacterium* sp. was isolated. Feeding studies conducted with chickens and rats using hay extract and pellets made from the nematode-infested hay demonstrated that toxicity was associated with an alcohol-soluble substance (Galloway 1961). Kurochkina and Chizhov (1980) in Russia reported animal poisoning after feeding on seed galls of *Anguina agrostis* from *Agrostis* sp. and suggested an association with *R. toxicus* or a related bacterium producing similar toxins.

Galey et al. (1997) described the occurrence of "staggers" in cattle in the central valley of California. Incidence ranged from 5 to 50% of animals, and the clinical course and syndrome resembled flood plain staggers. Investigation of three ranches where the losses occurred revealed the pastures did not contain perennial grasses usually associated with staggers, but they did contain other annual

grasses including ryegrass. There was no mention of examining the grasses for seed gall nematodes or bacterial head blight.

Riley et al. (2004a) examined bacterial galls from old Chewings fescue samples produced in New Zealand and New Jersey and concluded that the most likely cause of the livestock symptoms was the presence of corynetoxin-like toxins produced by a variant of *R. toxicus*. This conclusion was based on (i) detection of *R. toxicus* in galls from New Zealand, but not the United States, using a monoclonal antibody specific for *R. toxicus*; (ii) detection of corynetoxin-like chemicals by enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) in galls from both New Zealand and the United States, and in vitro inhibition of an enzyme specifically inhibited by corynetoxins and related tunicaminylluracil antibiotics by extracts of each sample (Jago et al. 1983); and (iii) the toxin mixture in both samples was very similar, but differed from that typical of *R. toxicus* galls (Anderton et al. 2004).

THE RATHAYIBACTER SPECIES

Investigations of bacterial head blight of grasses, their causal agents, and the vectors involved with them, now known as *Rathayibacter* spp. and *Anguina* or *Afrina* spp. (B. L. Barrantes-Infante, B. K. Schroeder, S. A. Subbotin, and T. D. Murray, unpublished data), respectively, began in 1897 when Emerich Rathay described bacteriosis disease of orchard grass (*Dactylis glomerata* L.) in Germany and associated a bacterium with it (Rathay 1899; Smith 1914). *Rathayibacter* species historically were placed in *Corynebacterium* (Collins and Bradbury 1986), and later in *Clavibacter* (Davis et al. 1984). The genus *Corynebacterium* originally contained Gram-positive, bacilliform-shaped bacteria (Fig. 3A) with 2, 4-diaminobutyric acid (DAB) in their cell walls (Carlson and Vidaver 1982; Collins and Bradbury 1986; Zgurskaya et al. 1993); however, since this was a heterogeneous genus (Carlson and Vidaver 1982; Dye and Kemp 1977; Starr et al. 1975), Davis et al. (1984) proposed establishing the genus *Clavibacter* for the Gram-positive, DAB-containing plant-pathogenic bacteria (Zgurskaya et al. 1993). Plant pathogenicity was considered a distinguishing characteristic of the bacteria. Based on morphological and physiological characteristics including menaquinone composition, along with phylogenetic analyses resulted in placement of some coryneform species in separate genera (Stackebrandt et al. 1988). Zgurskaya et al. (1993) subsequently proposed moving species that contain B2γ peptidoglycan with DAB in the cell wall (Sasaki et al. 1998), have MK-10 as the major menaquinone, phosphatidylglycerol

FIGURE 3

Scanning microscopic photo of **A**, *Rathayibacter* "agropyri" and **B**, infective juvenile of a seed gall nematode covered by *Rathayibacter* sp. (CA32). **A** and **B**, Scale bars = 1 and 20 μ m.

and diphosphatidylglycerol as the major phospholipids, saturated anteiso-15:0, anteiso-17:0, and iso-16:0 as the major branched fatty acids, and rhamnose and mannose as the major cell wall sugars to the genus *Rathayibacter*.

The genus *Rathayibacter* currently contains six valid species: *R. rathayi* (Smith 1913) Zgurskaya et al. 1993; *R. tritici* (Carlson and Vidaver 1982) Zgurskaya et al. 1993; *R. iranica* (Carlson and Vidaver 1982) Zgurskaya et al. 1993; *R. toxicus*; *R. caricis* Dorofeeva et al. 2002; and *R. festucae* Dorofeeva et al. 2002. Three other species have been identified but not validly described: *R. "agropyri"* (B. K. Schroeder, W. L. Schneider, D. G. Luster, A. Sechler, and T. D. Murray, unpublished data), *Rathayibacter* sp. EV (Riley et al. 2004b), and *Rathayibacter* sp. TV (Vasilenko et al. 2016). All *Rathayibacter* species identified and described to date are vectored by plant parasitic nematodes, a feature unusual among phytopathogenic bacteria.

R. toxicus occupies a basal position in the genus phylogeny and it is most distinct from the other species based on the 16S rRNA gene sequence (Fig. 4) (Dorofeeva et al. 2002; Wellington 2009). Based on preliminary data, we expect additional *Rathayibacter* species to be described in the near future (B. K. Schroeder, W. L. Schneider, D. G. Luster, A. Sechler, and T. D. Murray, unpublished data) (Starodumova et al. 2014) (Fig. 3).

***Rathayibacter rathayi*.** Ráthay (1899) initially discovered cocksfoot grass, also known as orchard grass, exhibiting gummosis near Vienna in 1897, but never observed similar symptoms on other grass species growing in the vicinity of the infected plants. He described affected plants as being somewhat stunted and covered with a thick, lemon-yellow bacterial slime on the uppermost stems and leaves, and inflorescences (Smith 1914), a symptom associated with many of the diseases caused by bacteria in this genus. Many organisms were isolated from the slime including fungi and a bacterium with lemon-yellow colonies. Although Ráthay inoculated seedlings and mature orchard grass plants without producing infection, he was convinced that the bacterium caused the disease. Smith (1913) later described the causal bacterium as *Aplanobacter*

rathayi in honor of Ráthay's pioneering work (Smith 1914); it is the type species for *Rathayibacter* (Burkholder 1948). This disease, commonly referred to as Rathay's disease, was frequently found throughout the European continent and England and is associated with seed as a source of inoculum (Dowson and d'Oliveira 1935). Rathay's disease is present and increasing in frequency in Oregon (Alderman et al. 2003, 2005).

***Rathayibacter "agropyri"*.** Around the same time as Ráthay's work, O'Gara (1915) described a bacterial disease of western wheatgrass (*Agropyron smithii* Rydb.) in the Salt Lake Valley, Utah with similar characteristics to those described on orchard grass by Ráthay (1899). He subsequently described the bacterium as *Aplanobacter agropyri*, suggesting yellow gum disease as the common name. O'Gara (1916) noted the absence of symptoms on other grass species in mixed stands and speculated that the bacterium may be spread by insects. In 1980, with no known type strain in existence and the inability to distinguish it from *R. rathayi* (syn. *Corynebacterium rathayi*), *R. "agropyri"* (syn. *Corynebacterium agropyri*) was not included on the Approved List of Bacterial Names (Skerman et al. 1980). Cultures resembling *R. "agropyri"* were isolated in 1982 from grass samples collected in the 1940s and 1950s and from a sample of wheatgrass with yellow EPS (Fig. 1D) collected in Montana in 1986 (Murray 1986). These cultures, which were stored at the Washington State University Mycological Herbarium, Pullman, WA, were recently characterized physiologically and molecularly. The name *R. "agropyri"* has been proposed to reestablish it as a valid species (B. K. Schroeder, W. L. Schneider, D. G. Luster, A. Sechler, and T. D. Murray, unpublished data).

***Rathayibacter tritici*.** Hutchinson (1917) described a bacterial disease (hereafter referred to as head blight) of wheat in the Punjab of India that resembled the diseases described by Ráthay and O'Gara, and which was known locally as tannan or tandu (also known as tundu). The disease was known prior to 1908, but was not of major concern because it affected a small percentage of plants within fields. Based on comments by D. Milne, economic botanist

FIGURE 4

Phylogenetic relationships within phytopathogenic *Rathayibacter* species (isolate, GenBank accession number): Bayesian 50% majority rule consensus tree from two runs as inferred from the analysis of the 16S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 60% are given for appropriate clades. Strains used in the analysis include the following: *R. "agropyri"* strain CA-4, *R. caricis*, *R. festucae*, *R. iranica*, *R. rathayi*, *Rathayibacter* sp. TV strain VKM Ac-2596, *R. toxicus* (D84127), *R. tritici*, *Rathayibacter* sp. Leaf296, *Rathayibacter* sp. EV strain FH238, *Rathayibacter* sp. strain CA36, and *Rathayibacter* sp. strain CA32. The outgroups were *Agromyces brachium*, *Agromyces rhizosphaerae*, and *Agromyces ramosus*.

in the Punjab at that time, Hutchinson noted the possible association of head blight with “eelworm” (nematode) damage, since it only occurred in areas of the field where they occurred. Hutchinson (1917) described the bacterium as *Bacterium tritici* (syn. *Pseudomonas tritici*), but was unable to successfully infect plants with the bacterium. Milne (1919) later described earcockle disease of wheat caused by the nematode *Tylenchus scandens* (= *T. tritici*; now *Anguina tritici* (Steinbuch 1799) Chitwood 1935). He noted that some plants infected with the nematode had distorted heads and produced a slimy yellowish substance, but did not mention a bacterium associated with it.

Fahmy and Mikhail (1925) described the occurrence and distribution of head blight of wheat caused by *Pseudomonas tritici* in Egypt. It was first reported to them in 1919, but was likely present before and believed to be introduced to Egypt on seed from India. Based on a survey in 1923, Fahmy and Mikhail (1925) observed that affected fields often were in close proximity to one another and that damage within fields was clustered in patches. They also observed that the disease did not spread aerially between fields since healthy and diseased fields were sometimes adjacent to one another, which led them to consider seed as the source of infection. Fahmy and Mikhail (1925) described the early symptoms of the disease as wrinkled or twisted leaves appearing on lower leaves on the wheat plant with a bright yellow gummy substance now known to be EPS exuding from these impacted plant structures. As the EPS dried, it became a solid, almost clear droplet on the plant surface (Fig. 1C). Fahmy and Mikhail (1925) further described the association of head blight with nematode damage (*Tylenchus tritici*) and demonstrated experimentally that the nematode was required to carry the bacterium into the plant for infection, although experimental conditions and data were not presented.

Carne (1926) described the occurrence of earcockle and head blight of wheat caused by *Pseudomonas tritici* in Western Australia. He noted that earcockle was first reported in 1898, but that head blight was not previously known in Western Australia. He also observed that earcockle did not form on plants with head blight, suggesting that nematodes were the agent of their own destruction. Based on field experiments, Carne (1926) concluded that the nematodes do not travel more than a few inches from the galls in soil and that the bacteria require the nematode to be carried into the plant and cause disease.

***Rathayibacter iranicus*.** *R. iranicus* was originally isolated from diseased wheat heads in Iran exhibiting symptoms characteristic of *Rathayibacter* infection of grass heads (Scharif 1961). The bacterium was isolated from the honey-yellow slime from aborted ovaries within infected wheat grains, but had discrepancies in physiological characteristics compared with *R. rathayi* (*Corynebacterium rathayi*) (Burkholder 1948, Dowson 1942; Sabet 1954), *R. “agropyri”* (*Corynebacterium agropyri*) (O’Gara 1915, 1916), and *R. tritici* (Burkholder 1948); consequently, it was described as a new species, *Corynebacterium iranicum* (Scharif 1961), now *R. iranicus* (Zgurskaya et al. 1993). This pathogen appears to be extremely limited in geographic distribution, having only been identified on wheat in Iran in 1961 and more recently in Turkey in 2009 (Postnikova et al. 2009).

***Rathayibacter toxicus*.** Bird and Stynes (1977) concluded that the bacterial pathogen associated with ARGV belonged to the *Corynebacterium rathayi*, *Corynebacterium agropyri*, and *Corynebacterium tritici* group based on colony color and symptomatology on the host plant, but there wasn’t enough data for a full comparison and species designation. Although presumptively referred to as *Corynebacterium rathayi* in many reports (Riley 1987), Riley and Ophel (1992) described the bacterium as *Clavibacter toxicus* sp. nov. based on serological properties, allozyme electrophoretic mobility, bacteriophage sensitivity, adhesion to seed gall nematodes, biochemical tests, DNA base composition, and growth factor requirements. Zgurskaya et al.

(1993) later erected the genus *Rathayibacter* and transferred *Corynebacterium iranicus*, *Corynebacterium rathayi*, *Corynebacterium tritici*, and six strains isolated from annual grasses in Australia that resembled *Clavibacter toxicus* to it. Sasaki et al. (1998) subsequently proposed *R. toxicus* comb. nov. on the basis of cell wall peptidoglycan content.

***Rathayibacter caricis*.** *R. caricis* was isolated from the phyllosphere of *Carex* sp. growing in the Central Chernozem Nature Park, Belgorod region, Russia (Dorofeeva et al. 2002). Although *R. caricis* was not found to be associated with any nematodes in this habitat, their association cannot be excluded. A potential vector for this bacterium could be *Heteroanguina caricis* (Solovyova and Krall 1983) Chizhov and Subbotin 1985, which induces leaf galls on several *Carex* species and is widely distributed in Estonia, Latvia, and Lithuania, as well as several regions of Northwest Russia. Recently, Starodumova et al. (2014) reported *R. caricis* from leaf galls induced by *Mesoanguina picridis* (Kirjanova 1944) Chizhov & Subbotin 1985 in *Acroptilon repens* (L.) DC. from Uzbekistan.

***Rathayibacter festucae*.** *R. festucae* was isolated and described by Dorofeeva et al. (2002) from small leaf galls on *Festuca rubra* L. infected by the nematode *Anguina graminis* (Hardy 1850) Filipjev 1936, collected in the Moscow region of Russia. This nematode parasitizes several *Festuca* species in Europe and northwestern European part of Russia.

***Rathayibacter* sp. EV.** *Rathayibacter* sp. EV was reported in 2004 by Riley et al. (2004b), who observed gummosis and leaf galls caused by *Anguina woodi* Swart et al. 2004 on *Ehrharta villosa* Schult. f. var. *villosa* in South Africa. The bacterium isolated from these plants was identified as a species of *Rathayibacter* based on physiological characteristics. However, DNA analysis of the bacteria demonstrated only 70% homology with genomic DNA from other *Rathayibacter* species, suggesting the isolate was a new species. The bacterium adheres to juveniles of *Anguina woodi*, the leaf gall nematode of *E. villosa* var. *villosa*, but not to *Anguina funesta* or *Anguina tritici* (Riley et al. 2004b). Interestingly, in planta production of toxin was detected in a bioassay and this work suggests that the bacterium is a unique and previously undescribed species currently referred to as *Rathayibacter* sp. EV (Riley et al. 2004b).

***Rathayibacter* sp. TV.** *Rathayibacter* sp. TV was isolated from leaves of *Tanacetum vulgare* L. infected with nematodes in the Main Botanical Garden, Moscow, Russia, and a draft genome sequence of this undescribed species was released in 2016 (Vasilenko et al. 2016). Although it had 99.6% 16S rRNA gene sequence similarity with *R. rathayi*, *R. iranicus*, and *R. tritici*, the results from matrix-assisted laser desorption ionization–time of flight mass spectra clustering and multilocus phylogenetic analysis (*gyrB*, *recA*, *rpoB*, and *ppk*) suggested that it was a novel species (Vasilenko et al. 2016). This was the first report of a *Rathayibacter* species associated with the foliar nematode *Aphelenchoides fragariae* (Ritzema Bos 1891) Christie 1932, which belongs to the nematode order Aphelenchida, family Aphelenchoididae, whereas all other previously known nematode vectors of *Rathayibacter* species belong to the order Tylenchida, family Anguinidae. This is significant because *Aphelenchoides fragariae* is an important parasite of several agricultural crops and numerous ornamentals with worldwide distribution.

Even though it is uncommon for a phytopathogenic bacterium to be vectored by a nematode, it should be noted that *Rathayibacter* species are not the only bacteria inhabiting nematode galls (Evtushenko et al. 1994). Several other members of the family Microbacteriaceae have been characterized and described: *Leifsonia poae* (Evtushenko et al. 2000) from root galls induced by the grass root gall nematode *Subanguina radicola* (Greeff 1872) Paramanov 1968 on *Poa annua* L. (Evtushenko et al. 2000); *Agreia bicolorata* Evtushenko et al. 2001 and *Agreia* spp. from leaf galls induced by *Heteroanguina graminophila* Evtushenko et al. 2001 on narrow reed grass, *Calamagrostis neglecta* (Ehrh.) Gaertn., B. Mey.

& Scherb. (Evtushenko et al. 2001; Starodumova et al. 2015); and three undescribed species of *Plantibacter* isolated from plant galls induced by *Anguina agrostis*, *Aplanobacter agropyri* Kirjanova 1955, and *Mesoanguina picridis* (Evtushenko and Takeuchi 2006).

NEMATODE VECTORS

The gall-forming nematodes of the subfamily Anguininae are obligate, highly specialized plant parasites that induce galls in various plant organs. Over 40 nominal species of gall-forming nematodes have been described. Three species, *Anguina tritici*, *Anguina agrostis*, and *Anguina funesta*, induce seed galls on some cereals and grasses, and are considered of economic importance as agricultural and quarantine pests in some countries (Chizhov and Subbotin 1990; Krall 1991). Several *Anguina* species are currently recognized as vectors of *Rathayibacter* species. *Rathayibacter tritici* and *R. iranicus* are exclusively associated with *Anguina tritici*; *R. toxicus* with *Anguina funesta* and *Anguina paludicola* Bertozzi & Davies 2009 (Bertozzi and Davies 2009; McKay et al. 1993; Riley and Ophel 1992); *R. rathayi* with *Anguina* sp. parasitizing *Dactylis glomerata* L. (Hardison 1945); and *R. festucae* with *Anguina graminis*. Cross-inoculation experiments and in vitro adhesion tests revealed that *Anguina australis* Steiner 1940, *Anguina tritici* and an *Anguina* sp. from *Holcus lanatus* L. are potential vectors of some strains of *R. toxicus* (Riley 1992; Riley and McKay 1990; Riley et al. 2001).

Little is known about the relationships between the *Rathayibacter* sp. and anguinid nematodes. Based on current information, these relationships should not be considered mutually beneficial since nematodes do not always remain viable in bacterial galls. Infective juvenile nematodes carry bacteria on their cuticle (Fig. 3B) in a film of water from galls resting on the soil surface to the growing points of a nearby young plant. The juvenile nematode (Fig. 5A) enters developing ovules, where they induce seed gall formation that the bacteria later colonize. Results of experiments

and observations suggest that the bacteria are not able to invade plants in the absence of nematodes, but advantages of the bacteria to the nematodes are not evident; in fact, they may be negative. For example, juvenile nematodes with attached bacteria moved significantly slower than nematodes without attached bacteria and thus, have a lower chance of successfully invading a plant (Bird and Riddle 1984).

Anguina funesta and *Anguina paludicola* are the two known natural nematode vectors of *R. toxicus*. The seed gall nematode *Anguina funesta* was first described in 1973 as *Anguina lolli* in the Ph.D. thesis by Price, and later named *Anguina funesta* when it was formally described (Price et al. 1979b). *Anguina funesta* was considered a synonym of *Anguina agrostis* by several authors; however, Powers et al. (2001) and Subbotin et al. (2004) examined relationships within *Anguina* using internal transcribed spacer (ITS) rRNA gene sequences and concluded that *Anguina funesta* was clearly different from *Anguina agrostis* and other described species and, thus, the validity of species was confirmed. *Anguina funesta* induces seed galls in the ovaries of some *Festuca*, *Lolium*, and *Vulpia* species (Price et al. 1979b; Riley 1995), but only *L. rigidum* and *V. myuros* (L.) C.C. Gmel. are known as hosts from natural infestations. ARGT is most common in South Australia and Western Australia, but also occurs in New South Wales. It spread to South Africa, most likely in infested seed from Australia, but did not spread as quickly or have as much economic impact (Subbotin and Riley 2012). Recently, *Anguina funesta* was also found in Oregon (Meng et al. 2012).

The life cycle of *Anguina funesta* is typical of other seed gall nematodes having one generation per year. The second stage juveniles (Fig. 5A) become dormant in drying galls, which drop to the soil surface near as plants senesce and survive anhydrobiotically until the following season. With rain, juveniles rehydrate, regain activity, and invade nearby plants. The juveniles congregate at the meristem of infested tillers and wait for ovary initiation before initiating a gall by invading the ovary and modifying its development.

FIGURE 5

Light microscopic photo of **A**, infective juvenile and **B**, female of seed gall nematode parasitizing *Sporobolus cryptandrus* in Idaho. Scale bar = 50 μ m.

Within the gall, second stage juveniles feed and molt through two further juvenile stages to become adults (Fig. 5B) (Riley and Barbeti 2008). Females and males usually range from a few up to 20 per gall (Riley and Bertozzi 2004). Each female can produce nearly 1,000 eggs (Price et al. 1979a, b). The nematodes develop to second stage juveniles inside the eggs and then hatch in the gall. The freshly hatched juveniles cannot survive desiccation, but mature physiologically to become the survival state as the host senesces and desiccates (Riley and Barbeti 2008).

Anguina paludicola, another seed gall nematode vectoring *R. toxicus*, was described by Bertozzi and Davies (2009) from two hosts: its type host, the nonnative grass *Polypogon monspeliensis*, and a native Australian grass, *Lachnagrostis filiformis* (Forst.) Trinius from areas subject to occasional seasonal flooding in South Australia and New South Wales. The life cycle and relationship of *Anguina paludicola* is similar to that of *Anguina funesta* but with some differences that represent adaptations for survival in ecosystems subject to seasonal inundation (Bertozzi and McKay 1995; Davis et al. 1995; McKay et al. 1993; Riley and Barbeti 2008).

SURVIVAL POTENTIAL

The production of gummosis or bacterial slime is associated with symptoms resulting from infection by *Rathayibacter*. This gummosis is EPS, composed of homopolysaccharides and heteropolysaccharides secreted by the bacteria (Donot et al. 2012; Leigh and Coplin 1992). It is associated with the cell wall, forming a capsule, or it may become detached, forming a slime layer (Coplin and Cook 1990; Denny 1995; Fett 1993). *R. "agropyri"* was isolated in 1982 from samples stored at the Washington State University Mycological Herbarium, Pullman, WA that were collected in the 1940s and 1950s (Murray 1986). This is the longest known survival of bacteria on a plant sample and it is hypothesized that survival was facilitated by the encapsulation of *R. "agropyri"* in the dried EPS, which enables the bacteria to withstand fluctuating environmental conditions (Boles et al. 2004; Franklin et al. 2011).

The seed gall nematode vectors of *Rathayibacter* spp. feed and reproduce within the developing ovaries of plant seeds and overwinter in seed galls as anhydrobiotic juveniles or adults. This dormant stage can survive within the seed gall for many years. For example, *Anguina tritici* did not lose its ability to invade wheat seedlings after 32 years of dormancy (Limber 1973). In this dehydrated state, nematodes are more tolerant to extreme environmental conditions than are their hydrated counterparts. The second stage juveniles of *Anguina agrostis* in seed galls survived exposure up to 155°C for 5 min, higher than that recorded for any other metazoan (Eisenback et al. 2013).

TOXIN PRODUCTION

The production of toxin in forage grasses is the result of a complex interaction between *Rathayibacter* species, a seed-gall nematode, a susceptible host, and potentially a bacteriophage. To date one species, *R. toxicus*, has been definitively shown to produce toxin, and two other species, *R. iranicus* and *Rathayibacter* sp. EV, are suspected toxin producers. The infection cycle begins when the bacterium adheres to the cuticle of compatible juvenile nematodes it encounters in the soil, and is carried to the growing point of the forage grass by the nematode (Riley and McKay 1990). The nematode initiates gall production as a natural part of the life cycle and *R. toxicus* competes with the nematode for the gall. The trigger for toxin production is unknown, but toxin generally appears late in the growing season as seeds are senescing. Senesced seeds dry and fall to the ground with galls colonized by bacteria to repeat the disease cycle.

The toxins produced by *R. toxicus*, referred to as corynetoxins, are a mixture of chemically related glycolipids belonging to the

tunicamycin group of nucleoside antibiotics (Eckardt 1983) that prevent N-linked glycosylation resulting in cell death. The exact mechanisms of toxin production remain unknown, but related systems in the *Actinomycetes* such as *Streptomyces chartreusis* Leach et al. 1953, *S. clavuligerus* Higgins and Kastner 1971, and *Actinosynnema mirum* Hasegawa et al. 1978 synthesize tunicamycin in a multistep biosynthetic pathway involving uridine, UDP-GlcNAc, and at least five enzymes (Price and Tsvetanova 2007; Wyszynski et al. 2010).

Previous reports implicate corynetoxin production in *R. toxicus* with the presence of a bacteriophage (Ophel et al. 1993). Stynes and Bird (1983) originally identified a bacteriophage commonly associated with *R. toxicus* by using electron microscopy. Initial reports indicated that adding the bacteriophage (NCPBP 3778) to cultures of *R. toxicus* consistently resulted in the production of tunicamycin (Ophel et al. 1993). However, later work identified toxin-producing cultures of *R. toxicus* devoid of the bacteriophage (Kowalski et al. 2007). The ultimate role of bacteriophage infection in *R. toxicus* toxin production in nature remains to be determined, although it should be noted that a counterpart phage has not been identified for *R. iranicus* or *Rathayibacter* sp. EV system. It is unclear whether toxin production is a product of the phage, the bacteria, or an interaction/combination of the two. A complete genome sequence of the phage is available, but no genes related to toxin production were identified as part of the phage DNA (W. Schneider, A. Sechler, and E. Rogers, unpublished data).

The evolutionary drivers of developing and maintaining toxin production in *R. toxicus* are also unclear. Tunicamycin serves as a very effective nematicide, but most members of the genus are able to overcome nematodes and usurp galls without toxin production. In addition, there is a fitness cost to toxin production, as *R. toxicus* cultures grow slower while producing tunicamycin (Ophel et al. 1993). The toxin may serve multiple purposes, acting as a weapon against competing bacteria and other microorganisms, while also eliminating nematodes from galls. However, this strategy appears to be limited to *R. toxicus* and possibly *R. iranicus* and *Rathayibacter* sp. EV. The triggering of toxin production by the bacteriophage is also interesting. In this case the bacteriophage may have evolved a mechanism for triggering toxin production to increase the success of its primary obligate host.

DETECTION AND DIAGNOSTICS FOR BACTERIA, NEMATODE, AND TOXIN

Methods for specifically detecting *R. toxicus* in infested plants have been published (see below), and several full genomic sequences are available in open access databases. However, the Select Agent status and associated scarcity of biological samples has hampered the validation of a diagnostic assay for official confirmation of the presence of the pathogen in the United States.

Sampling. As with most field-oriented plant pathogen diagnostic methods, the primary challenges are in sampling and sample preparation. *R. toxicus* infests forage grasses that may be spread across several acres; samples may consist of hay or seed from areas where symptomatic animals are identified. The most common methods used in Australia involve soaking seed, infested grass or hay samples in water or buffer and recovering live bacteria for subsequent analyses. The degree of infestation influences the sensitivity of the diagnostic assay. Larger samples result in less certainty with regard to diagnosis of the causal agent.

Morphological and biochemical traits. Like most bacteria, *Rathayibacter* spp. can be identified to the genus level by a combination of morphological characters, cell wall components and carbon substrate utilization (Burkholder 1948; Evtushenko and Takeuchi 2006; Zgurskaya et al. 1993). Further distinction may be achieved for some species by including specific traits such as salt

intolerance (Riley and Ophel 1992), bacteriocin production (Gross and Vidaver 1979) and vancomycin resistance (inferred by presence of *vanA* gene) (Postnikova et al. 2017) (A. Sechler, unpublished data). *Rathayibacter* spp. are not difficult to isolate, but grow slowly, and in general more rapid methods are required for diagnosis in order to respond quickly to a disease outbreak.

Nucleic acid-based assays: Bacteria. Although time-consuming, amplified fragment length polymorphism (Agarkova et al. 2006) and PCR-16S rRNA-RFLP (Lee et al. 1997) analyses have been used to characterize the phylogeny of *Clavibacter* and *Rathayibacter*, and could be used to identify *Rathayibacter* spp. More rapid assays using methods such as PCR are preferred to accommodate smaller sample size and the ability to rapidly and accurately screen large numbers of samples. *Rathayibacter* spp. can be identified by PCR of 16S rRNA genes from samples containing live bacteria, followed by sequencing and searches against gene databases. A PCR assay based on the *R. toxicus* 16S rRNA region V9 and bacteriophage NCPPB 3778 sequence analysis was developed for detection of *R. toxicus* and the associated phage (Kowalski et al. 2007). However, only one other species in the genus, *R. tritici*, was used to test whether the target sequence was unique to *R. toxicus*. PCR/mass spectrometry was applied to detect and identify multiple species of plant pathogenic bacteria, including *R. iranicus*, *R. rathayi*, *R. tritici*, and *R. toxicus* (Postnikova et al. 2008). More recently, real-time PCR primers and probes were described to identify the same species from grasses and grains (Postnikova et al. 2017).

The analysis of vancomycin resistant protein *vanA*, CRISPR-associated protein *cse4*, *secA* ATPase, chromosome partition protein SMC, tRNA dihydrouridine synthase, and cysteine desulfurase genes and inter-simple sequence repeats allowed identification of three population groups of *R. toxicus* (Arif et al. 2016). Sequences of the *gyrB*, *recA*, *rpoB*, and *ppk* genes also could be useful for identification of *Rathayibacter* species (B. K. Schroeder, W. L. Schneider, D. G. Luster, A. Sechler, and T. D. Murray, unpublished data) (Starodumova et al. 2014; Vasilenko et al. 2016).

Nucleic acid-based assays: Nematode. Diagnosis of the gall-forming nematodes based on morphological and morphometrical characters is difficult, time consuming, and requires significant expertise. Powers et al. (2001) and Subbotin et al. (2004) molecularly characterized agriculturally important anguinid species and showed the ITS rRNA gene could serve as a reliable marker for identification of this nematode group. Powers et al. (2001) also showed that PCR-ITS1-RFLP using *AluI*, *BsrI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, and *TaqI* could clearly distinguish *Anguina agrostis*, *Anguina funesta*, *Anguina pacifica* Cid del Prado Vera & Maggenti 1984, *Anguina tritici*, *A. agropyronifloris* Norton 1965, *Anguina microlanae* (Fawcett 1938) Steiner 1940, *Afrina wevelli* van den Berg 1985, and several unidentified species from one another. Later, PCR-ITS1-RFLP and sequencing of this gene region was used to confirm the presence of *Anguina funesta* in Oregon (Meng et al. 2012). Ma et al. (2011) developed TaqMan real-time PCR methods for detection of second stage juveniles of *Anguina agrostis*. Using the ITS rRNA gene fragment, Li et al. (2015) developed and validated TaqMan real-time PCR methods for detection of *Anguina agrostis*, *Anguina funesta*, *Anguina tritici*, and *Anguina pacifica*. Although all these protocols enable fast and accurate identification of these species, comparative sequence analysis of the ITS region should be considered as the main approach if test results are inconclusive.

Immunoassays: Bacteria. An ELISA for detection of *R. toxicus* was developed against a surface antigen from the bacterium with subsequent improvements to sampling and streamlining the assay (Masters et al. 2006; 2011). A semiquantitative ELISA protocol was later developed for improved risk evaluation (Masters et al. 2014). The ELISA protocols and improvements have been applied to identify pastures at high-risk for ARG, and to screen exports in Australia since 1996. The monoclonal antibodies raised against the *R. toxicus* antigen were screened for specificity against *R. tritici*,

R. rathayi, *R. michiganense* subsp. *insidiosum* (sic: *Clavibacter michiganensis* subsp. *insidiosus*), and *Corynebacterium agropyri* (*R. "agropyri"*). The improved immunoassay for *R. toxicus* in hay (Masters et al. 2011) is used by Diagnostics and Laboratory Services, Biosecurity and Regulation Department of Agriculture and Food, Western Australia (DAFWA) and the laboratories at the South Australian Research and Development Institute (SARDI) (A. Masters, personal communication).

Immunoassays: Corynetoxins. Although the corynetoxin (tunicamycin antibiotic) responsible for ARG can be identified and detected through chemical analyses such as HPLC and mass spectrometry, these techniques are expensive, time-consuming, and require large and impractical amounts of sample material. A standardized protocol using ELISA was developed with monoclonal antibodies raised against conjugated tunicamycin for more rapid and sensitive toxin identification in hay and veterinary samples (Than et al. 2002). However, this assay is not currently in use by any government.

DISEASE MANAGEMENT

It is theoretically possible to control ARG at any level of the interaction: grazing animal, grass species, nematode, or bacterium (Fisher 1977). It is generally not practical to apply nematicides or antibiotics to large grazing areas to control the nematode or bacterium directly. In an attempt to immunize animals against tunicamycin toxicity, researchers at the CSIRO Division of Animal Health in Australia injected sheep with tunicamycin conjugated to a carrier protein (Than et al. 1998). Although the treatment provided protection against ARG after consumption of contaminated seed heads in approximately 90% of vaccinated animals, this vaccine does not appear to have been commercialized. Likewise, an antidote was developed, but never commercialized (Riley et al. 2014); in both cases the practicality of administering the treatment and their effectiveness against high doses of toxin were questionable. Furthermore, the need for control measures has declined over the past 30 years due to a decline in ARG outbreaks, largely owing to success of past management practices including deployment of the antagonist *Dilophospora alopecuri* (Fr.) Fr. (Riley 1994).

Currently used control measures focus on preventing ryegrass seed maturation by applying herbicide, mowing and/or heavy grazing immediately after head emergence, prior to seed set and maturation when tunicamycin toxicity increases rapidly (<https://www.agric.wa.gov.au/livestock-biosecurity/controlling-annual-ryegrass-toxicity-arg-through-management-ryegrass-pasture>). This approach must be followed for 2 to 3 years and combined with planting clean ryegrass seed to reduce the number of nematode and bacterial galls as well as the incidence of ARG.

LOOKING AHEAD

Although *R. toxicus* is a Select Agent in the United States, the other five described *Rathayibacter* species remain relatively unstudied because the diseases they cause are obscure and do not have a recognized economic impact. This situation has resulted in knowledge gaps regarding the basic biology of these plant pathogens. *R. toxicus* poses a threat to U.S. agriculture because of its ability to produce corynetoxins that are lethal to grazing animals. Two other species, *R. iranicus* and the undescribed *Rathayibacter* sp. EV are suspected of being able to produce corynetoxins. The tunicamycin biosynthetic pathway responsible for corynetoxin production is well characterized from other bacteria, but its regulation and evolutionary role in the life cycle of *R. toxicus* is not understood. A bacteriophage was initially implicated in toxin production by *R. toxicus*, but current evidence suggests that the association is not absolute, and there is no evidence for phage involvement in toxin production by *Rathayibacter* sp. EV or *R. iranicus*.

Little is known about the potential for toxin production by the other described *Rathayibacter* species. Reports of unexplained livestock poisonings in the United States leave open the possibility that *R. toxicus* or a related toxigenic *Rathayibacter* species may be responsible (Riley et al. 2003). These poisonings have occurred mostly in the western United States over a long period of time and do not have a clear pattern, which makes them very difficult to study. Preliminary data suggest additional species of both *Rathayibacter* and *Anguina* exist in the United States (B. L. Barrantes-Infante, unpublished data) (B. K. Schroeder, W. L. Schneider, D. G. Luster, A. Sechler, and T. D. Murray, unpublished data). Consequently, important data needed to conduct a risk analysis for establishment of *R. toxicus* or other toxigenic *Rathayibacter* species in the United States are missing and necessitates a need to develop a better understanding of the species of *Rathayibacter* and their vectors that are present in the United States. Development and validation of detection assays for *R. toxicus* and its vectors that can discriminate among the known and unknown *Rathayibacter* and their vectors also are needed to protect U.S. agriculture from its introduction. As new *Rathayibacter* and *Anguina* species are identified and characterized, existing detection assays need to be reevaluated to insure they remain specific for the target pathogen. It also will be necessary to determine if these new species are capable of producing corynetoxins now or if given the proper circumstances such as introduction of the corynetoxin biosynthetic gene cluster, could they produce corynetoxins? These are important questions given that the known *Rathayibacter* species produce similar disease symptoms in several grass species and are found in similar environments as *R. toxicus*.

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