Clavibacter toxicus sp. nov., the Bacterium Responsible for Annual Ryegrass Toxicity in Australia

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The *Clavibacter* sp. associated with annual ryegrass toxicity (ARGT) in Australia has been reported to differ from other *Clavibacter* spp. in serological properties, allozyme electrophoretic mobility, bacteriophage sensitivity, and adhesion to nematodes (*Anguina* spp.). To clarify its taxonomic position, eight strains of the ARGT *Clavibacter* sp. were compared with strains of *Arthrobacter ilicis*, *Clavibacter iranicum*, *Clavibacter michiganense*, *Clavibacter rathayi*, *Clavibacter tritici*, *Curtobacterium flaccumfaciens*, and *Rhodococcus fascians*. The ARGT strains were distinguishable from the other bacteria on the basis of the results of a range of biochemical tests. DNA base composition, growth factor requirements, and growth rates were also determined. A new species, *Clavibacter toxicus*, is proposed, with type strain CS14 (= ICMP 9525 = NCPPB 3552).

Annual ryegrass toxicity (ARGT) often results in fatal poisoning of livestock that graze on ryegrass (*Lolium rigidum* Gaudin) which is infected with a toxigenic *Clavibacter* sp. (1, 7, 18, 20). The bacterium is carried into the grass by *Anguina funesta* Price, Fisher, and Kerr 1979, a parasitic nematode of ryegrass (19, 25). ARGT was first recorded in 1956 at Black Springs, South Australia (9), but the involvement of a toxigenic bacterium was not recognized until 1968 (18). The bacterium was found to be related to a group of *Clavibacter* spp. that cause similar diseases in grasses and have been partially described (17). A morphological description was published in 1977 (1). The ARGT bacterium was referred to as *Corynebacterium* sp. and *Corynebacterium rathayi* [now *Clavibacter rathayi* (Smith 1913) Davis et al. 1984]. Identification as *Corynebacterium rathayi* was presumptive and was not based on results of comparative studies (20). Since *Corynebacterium rathayi* is a markedly different organism in culture and strains of the ARGT bacterium were not included in the work of Davis et al. (5) in which reclassification of *Corynebacterium rathayi* into a new genus, *Clavibacter*, was proposed, the position of the ARGT bacterium needed to be clarified. An immunological study indicated that the ARGT bacterium was distinct from *Corynebacterium* (*Clavibacter*) *rathayi* and other coryneform plant pathogens (20). The detection of 2,4-diaminobutyric acid in its cell walls confirmed that this organism belongs in the genus *Clavibacter*, and it was recommended that the bacterium be known as *Clavibacter* sp. (20).

Further studies have confirmed that the ARGT bacterium is distinct from other *Clavibacter* spp. Allelic profiles determined by allozyme electrophoresis of 20 enzymes indicated that the levels of genetic difference were at least 85% between the ARGT bacterium and the other *Clavibacter* spp. studied and 18% or less within the 11 strains of the ARGT bacterium (24). A bacteriophage which lysed all of the strains of the ARGT bacterium tested but no other *Clavibacter* sp. has been described (21). Studies of the adhesion of 35 plant-pathogenic coryneform strains (mostly *Clavibacter*

spp.) to seven nematode populations representing five *Anguina* spp. showed that there were significant variations in the adhesion patterns of the strains, and this finding supported with the separation of the ARGT bacterium from other *Clavibacter* spp. (22).

In this paper we propose that the ARGT bacterium should be named as a new species in the genus *Clavibacter*. The results of comparative physiological and biochemical tests supported this proposal.

MATERIALS AND METHODS

Bacterial strains. The strains which we used in this study and their origins are shown in Table 1. Our bacterial nomenclature is the same as that used by Davis (4) in his review of the taxonomy of plant-pathogenic coryneform bacteria. All of the strains were maintained on medium 523M, which contained (per liter) 10 g of sucrose, 2 g of Casamino Acids (Difco), 2 g of yeast extract (Difco), 2 g of K₂HPO₄, 0.3 g of MgSO₄ · 7H₂O, and 15 g of agar. The strains were grown at 26°C unless indicated otherwise. Cultures were kept in sterile glycerol at -20°C for medium-term storage and freeze-dried for long-term storage.

Biochemical tests. (i) **Growth and acid production from carbon sources.** Medium C of Dye and Kemp (6) was used to test growth on and production of acid from carbon sources. The following carbon sources were tested at concentrations of 0.5% (wt/vol): galactose, mannitol, and mannose (Ajax Chemicals, Sydney, Australia); ethanol, inulin, and xylose (BDH Chemicals, Port Fairy, Victoria, Australia); and dulcitol (galactitol), erythritol, adonitol (ribitol), and melezitose (Sigma). Tubes were incubated at 26°C and examined after 7, 14, 21, and 28 days for growth and acid production.

(ii) **Utilization of organic acids.** The modified YSA medium described by Dye and Kemp (6) was used to test utilization of sodium salts of succinate, tartrate, acetate, malonate, and glutamate (0.2%, wt/vol; BDH Chemicals). Tubes were incubated at 26°C and examined after 7, 14, 21, and 28 days.

(iii) **Maximum NaCl tolerance.** Medium YSB of Dye and Kemp (6) containing 0 to 10% (wt/vol) NaCl in 1% gradations was used to test NaCl tolerance. Cultures were shaker incubated at 25°C and examined for growth after 3, 7, and 14 days.

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TABLE 1. Strains used in this study

Taxon	Laboratory no.	Source ^a	Other designation(s) ^a
<i>Arthrobacter ilicis</i>	CS10 ^T	ICMP 2607	ATCC 14264 ^T , ICPB C1144 ^T , NCPPB 1228 ^T
<i>Clavibacter iranicum</i>	CS13 ^T	ICMP 3496	ICPB C1148, NCPPB 2253 ^T
<i>Clavibacter michiganense</i> subsp. <i>insidiosum</i>	CS20	NCPPB 1109	
<i>Clavibacter michiganense</i> subsp. <i>michiganense</i>	CS25 ^T	NCPPB 2979 ^T	
<i>Clavibacter michiganense</i> subsp. <i>nebraskense</i>	CS24 ^T	NCPPB 2581 ^T	
<i>Clavibacter rathayi</i>	CS5	ICMP 2571	ATCC 13659, ICPB CR1, NCPPB 80
	CS26 ^T	NCPPB 2980 ^T	ICMP 2574 ^T
<i>Clavibacter tritici</i>	CS4	ICMP 2624	ICPB CT106, NCPPB 255, NCTC 6255
	CS12	ICMP 2628	ICPB CT104
	CS21 ^T	NCPPB 1857 ^T	ATCC 11403 ^T , ICPB CT102 ^T
<i>Clavibacter</i> sp. (ARGT bacterium)	CS2	South Australia	
	CS3	South Australia	
	CS14 ^T	South Australia	ICMP 9525 ^T , NCPPB 3552 ^T
	CS28	WSM 188	ICMP 6307
	CS30	WSM 447	
	CS31	WSM 455	
	CS33	South Australia	ICMP 9526
	CS34	South Australia	ICMP 9527, NCPPB 3553
<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i>	CS8 ^T	ICMP 2584 ^T	NCPPB 1446 ^T
<i>Curtobacterium flaccumfaciens</i> pv. <i>poinsettiae</i>	CS19	NCPPB 854	
<i>Rhodococcus fascians</i>	CS27 ^T	NCPPB 3067 ^T	ATCC 12974 ^T , ICMP 5833 ^T

^a ATCC, American Type Culture Collection, Rockville, Md.; ICPB, International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis; ICMP, International Collection of Microorganisms from Plants (formerly Plant Diseases Division Culture Collection), Plant Diseases Division, Department of Scientific and Industrial Research, Auckland, New Zealand; NCPPB, National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Hatching Green, Harpenden, Hertfordshire, England; WSM, Western Australian Department of Agriculture, South Perth, Australia.

(iv) **H₂S production.** The method described by Fahy and Hayward (8) was used to test H₂S production from both 0.01% cysteine hydrochloride (Sigma) and 0.5% neutralized bacteriological peptone (Oxoid). Tubes containing 5 ml of medium were inoculated and incubated with a lead acetate strip suspended over the culture. Lead acetate strips were prepared by impregnating strips of filter paper with 5% lead acetate; the strips were then air dried and autoclaved. Cultures were examined after 7, 14, and 21 days of growth at 26°C.

(v) **Starch hydrolysis.** Starch hydrolysis was tested by spotting cultures onto YNA medium containing 5 g of yeast extract (Difco) per liter, 23 g of nutrient agar (Difco) per liter, and 0.2% soluble starch (Ajax Chemicals). After 7 to 14 days of incubation (depending on growth), the plates were flooded with an iodine solution containing 10 g of iodine per liter and 20 g of potassium iodide per liter.

(vi) **Growth at 37°C.** Bacteria were streaked onto 523M agar and incubated at 37 ± 0.5°C and at 26°C (as a positive control). The plates were examined for growth after 3, 7, and 14 days.

(vii) **Gelatin hydrolysis.** Duplicate tubes containing nutrient gelatin (Difco) were stab inoculated, and the tubes were assessed after 7, 14, 21, and 28 days of incubation at 26°C.

(viii) **Oxygen requirements.** Duplicate tubes containing 523M agar were stab inoculated, and after inoculation one tube of each pair was covered with 10 mm of sterile paraffin oil-petroleum jelly (1:1). Growth was recorded after 7 and 14 days.

(ix) **Other tests.** The methods of Dye and Kemp (6) were used to test catalase activity, nitrate reduction, production of acetoin, tyrosinase, and urease, and lipolysis of Tween 80 (Sigma) and cotton seed oil (Drug Houses of Australia). Oxidase activity was tested by using the methods described by Fahy and Hayward (8).

DNA base composition. DNAs from strains CS2 and CS14^T (T = type strain) were prepared from cultures grown in 200

ml of 523M broth by using the method described by Hunter (11). DNA base composition was determined from the thermal denaturation point as described by Marmur and Doty (14), using 25 µg of DNA per ml in PE buffer (10 mM phosphate buffer [pH 7.2], 1 mM EDTA). Denaturation profiles were measured by using a Pye & Unicam model 5P8-200 spectrophotometer equipped with an Accuron model SPX 876 temperature controller.

Growth factor requirements. The method used to determine growth factor requirements was modified from the method of Keane et al. (13). Bacteria from 523M agar plates were suspended in buffered saline, washed with sterile distilled water (10 min, 3,000 × g), and inoculated onto agar containing 0.5 g of NH₄H₂PO₄ per liter, 0.5 g of K₂HPO₄ per liter, 0.2 g of MgSO₄ · 7H₂O per liter, 5 g of NaCl per liter, and 1% (wt/vol) sucrose (autoclaved separately); this medium was amended with 0.1 µg of biotin per ml, 0.1 µg of nicotinic acid per ml, 0.2 µg of calcium pantothenate per ml, or 0.1% (wt/vol) yeast extract (Difco). Growth was assessed after 4, 7, 14, and 28 days of incubation at 26°C.

Growth rates. The growth rates of three ARGT strains, two strains of *Clavibacter tritici*, and two strains of *Clavibacter rathayi* were determined in 523M broth. Bacteria from 48-h-old cultures were added to 50 ml of broth to give an initial concentration of 1 × 10⁶ cells per ml. The cells were grown on a rotary shaker at 25°C, 3 ml of culture was removed for each sample, and the optical density at 600 nm was measured every 8 h for 6 days, by which time all of the cultures had reached saturation. The growth rates were plotted, and the time taken for each culture to grow from an optical density at 600 nm of 0.5 to an optical density at 600 nm of 1.0 was calculated.

RESULTS

Biochemical tests. The ARGT strains differed from all of the other species tested in their reactions in biochemical

TABLE 2. Biochemical characteristics of coryneform strains

Characteristic	ARGT bacterium (n = 8) ^a	<i>Clavibacter</i> <i>iranicum</i>	<i>Clavibacter</i> <i>rathayi</i> (n = 2)	<i>Clavibacter</i> <i>tritici</i> (n = 3)	<i>Clavibacter</i> <i>michigan-</i> <i>ense</i> subsp. <i>insidiosum</i>	<i>Clavibacter</i> <i>michigan-</i> <i>ense</i> subsp. <i>nebrask-</i> <i>ense</i>	<i>Clavibacter</i> <i>michigan-</i> <i>ense</i> subsp. <i>michigan-</i> <i>ense</i>	<i>Arthro-</i> <i>bacter</i> <i>ilicis</i>	<i>Curtobac-</i> <i>terium</i> <i>flaccum-</i> <i>faciens</i> pv. <i>flac-</i> <i>cum-</i> <i>faciens</i>	<i>Curtobac-</i> <i>terium</i> <i>flaccum-</i> <i>faciens</i> pv. <i>poin-</i> <i>settiae</i>	<i>Rhodo-</i> <i>coccus</i> <i>fascians</i>
Acid produced from:											
Adonitol	— ^b	—	—	—	—	—	—	—	+	+	—
Dulcitol	—	—	—	—	—	—	—	—	—	—	—
Erythritol	—	—	—	—	—	—	—	—	—	+	—
Ethanol	—	—	—	—	—	—	—	+	—	—	+
Galactose	+	+	+	+	+	+	+	+	+	+	+
Inulin	—	—	—	+	—	—	—	—	—	—	—
Mannitol	—	—	+	+	—	+	—	+	+	+	+
Mannose	+	+	—	+	+	+	+	+	+	+	+
Melezitose	—	+	—	—	—	—	—	+	+	+	—
Xylose	+(7)	+	+	+	+	+	+	+	+	+	+
Utilization of:											
Acetate	—	—	+(1)	+	—	+	+	+	+	+	+
Glutamate	—	—	—	+	—	—	+	+	+	—	+
Malonate	—	+	—	+	—	+	+	+	+	—	+
Succinate	—	+	+	+	—	+	+	+	+	—	+
Tartrate	—	—	—	—	—	+	+	—	—	—	+
Maximum NaCl concn tolerated (%)	1	2	3	4	3	5	5	5	8	8	6
Starch hydrolysis	—	+	+(1)	—(2)	—	+	+	—	+	+	—
Growth at 37°C	—	—	—	—	—	—	—	+	+	+	—
Gelatin hydrolysis	—	—	+	—	—	—	—	+	—	—	—
H ₂ S produced from:											
Cysteine	+	+	+	+	+	+	+	+	+	+	+
Peptone	—	+	+	+	—	+	+	+	—	—	+
Pigmentation ^c	Y	Y	Y	Y	W	O	Y	Y	Y	O	O

^a n is the number of strains tested. Where no number is given, only the type strain was tested.

^b +, positive reaction; —, negative reaction. Where strains differed in their reactions, the most common reaction is indicated, and the number of strains which gave that reaction is indicated in parentheses.

^c Y, yellow; O, orange; W, white.

tests (Table 2). The ARG T strains differed from the *Clavibacter tritici* strains in their carbon source and organic acid utilization patterns. All of the *Clavibacter tritici* strains produced acid from mannitol and inulin and utilized succinate, acetate, malonate, and glutamate. The *Clavibacter tritici* strains tolerated higher levels of NaCl (4%) in the growth medium than the ARG T strains did (1%). The ARG T strains also differed from the *Clavibacter rathayi* strains in acid production. The two *Clavibacter rathayi* strains produced acid from mannose and mannitol, and they utilized succinate. One of the *Clavibacter rathayi* strains tested utilized acetate, but none of the ARG T strains did. The *Clavibacter rathayi* strains tolerated higher concentrations of NaCl in the growth medium and hydrolyzed gelatin, which the ARG T strains did not. The ARG T strains also differed from the type strain of *Clavibacter iranicum* in a number of respects. The *Clavibacter iranicum* strain produced acid from melezitose and utilized malonate, which the ARG T strains did not. The *Clavibacter iranicum* type strain also tolerated a slightly higher level of NaCl in the growth medium (Table 2). All strains of *Clavibacter iranicum*, *Clavibacter rathayi*, and *Clavibacter tritici* produced H₂S from peptone, while the ARG T strains did not. The ARG T strains differed from the type strains of seven other coryneform plant-pathogenic bacterial species used in this study (Table 2).

In general, the ARG T strains were more discriminating in their utilization of carbon sources and organic acids. They

produced acid from only 3 of the 10 carbon sources tested and utilized none of the five organic acid sources tested. Tolerance of NaCl was low (1%), and none of the strains hydrolyzed starch or gelatin. All of the ARG T strains produced H₂S from cysteine but not from peptone. All of the ARG T strains produced catalase, and none produced oxidase. Tests for urease, tyrosinase, acetoin, lipolysis of Tween 80 and cotton seed oil, and nitrate reduction performed with the ARG T strains and the type strains of *Clavibacter rathayi*, *Clavibacter tritici*, *Clavibacter iranicum*, and *Clavibacter michiganense* subsp. *michiganense* were all negative except for acetoin production by *Clavibacter michiganense* subsp. *michiganense*. All of the ARG T strains had an obligate requirement for oxygen. Growth of the ARG T strains was very slow, and great care had to be taken in the tests to incubate the cultures for a sufficient length of time and to avoid contamination. All eight ARG T strains tested were uniform in their responses except that one strain, strain CS28, did not produce acid from xylose while the other seven ARG T strains did.

Most of the tests performed with the other coryneform strains produced results which agreed with the results previously published by Dye and Kemp (6) and Collins and Bradbury (3). However, there were 14 instances in which the results shown in Table 2 differed from those of Davis et al. (5). In nine of these cases our results agreed with those of Dye and Kemp (6), and in four cases our results differed from the results of both studies. We also observed a differ-

ence in acetate utilization between the two strains of *Clavibacter rathayi* tested. There are numerous differences in biochemical test results described in the previously published studies. Dye and Kemp noted that there were differences between strains of the same species and when some tests were repeated with a single strain. Consistent methodology for biochemical tests for this group of organisms seems to be difficult to achieve, and this observation highlights the diagnostic value of methods such as serology and bacteriophage sensitivity.

DNA base composition. The DNAs of strains CS2 and CS14^T contained 67.6 and 67.0 mol% guanine plus cytosine (G+C), respectively. These values fell in the previously published range for G+C contents of other *Clavibacter* spp. (i.e., 67 to 78 mol% [3]).

Growth factor requirement. All eight ARG^T strains which we tested required the addition of 0.1% yeast extract to the growth medium. Addition of biotin alone or of biotin, nicotinic acid, and calcium pantothenate together to the minimal medium did not satisfy the growth requirements. Both the type strain of *Clavibacter tritici* and the type strain of *Clavibacter rathayi* grew with the addition of biotin alone, and the type strain of *Clavibacter iranica* required the addition of biotin, calcium pantothenate, and nicotinic acid.

Growth rates. The ARG^T strains grew much more slowly than strains of either *Clavibacter rathayi* or *Clavibacter tritici*. The mean doubling time (the time taken to grow from an optical density of 0.5 to an optical density of 1.0) was 18 h for the ARG^T strains, compared with 9 h for *Clavibacter tritici* and 8 h for *Clavibacter rathayi*.

DISCUSSION

The bacterium associated with ARG^T has been shown to be a species of the genus *Clavibacter* and to be distinct from other *Clavibacter* spp. associated with vector nematodes (*Anguina* spp.) in grasses. It is distinct in its serology (20), allelic profiles (24), bacteriophage sensitivity (21), and the biochemical and physiological properties described in this paper. Also, the results of a preliminary investigation of the fatty acid profiles of two strains suggest that these organisms are significantly different from *Clavibacter rathayi* (26). There is now sufficient evidence and descriptive information to justify naming a new taxon.

Although the ARG^T bacterium is distinct from the other *Clavibacter* spp., in particular those of similar ecology, the strains which have been isolated are not homogeneous. An allozyme study (24) showed that there is 18% variation within this species. The greatest differences reported are in adhesion patterns (22); the strains isolated from South Australia and Western Australia differ particularly in their interactions with *Anguina* spp. other than the normal vector, *Anguina funesta*. Among the characteristics included in this study one strain differed in the utilization of xylose; otherwise, the reactions were homogeneous. Further investigation may reveal that subspecific or pathovar groupings of strains are justified.

Investigations of toxigenic bacteria that were recently isolated from *Agrostis avenacea* J. F. Gmelin (blowgrass) in northern New South Wales and from *Polypogon monspeliensis* (L.) Desf. (annual beardgrass) from the Stewart Range in southeast South Australia showed that these organisms are strains of the *Clavibacter* sp. described in this paper (15). Allozyme electrophoresis, an enzyme-linked immunosorbent assay, and bacteriophage sensitivity were used to identify these strains. The bacterial infections of

these grasses were associated with nematodes, were toxic to livestock, and occurred outside the known range for ARG^T and the distribution of *L. rigidum*. The only other reports of toxicity to livestock due to a bacterium associated with an *Anguina* sp. were from *Festuca rubra* subsp. *commutata* Gaudin in Oregon around 1960 (10, 12). The bacterium was not identified, but given that *Festuca* spp. are also hosts of *Anguina funesta* (17), it is possible that this bacterium was the same as the organism described here. The reports cited above highlight the need to formally name this bacterium; reference to it as "the *Clavibacter* sp. associated with ARG^T" is clearly inadequate.

The name which we propose is *Clavibacter toxicus*; this name was chosen because this organism produces corynetoxins (antibiotics belonging to the tunicamycin group) in colonized grasses (7) and is responsible for often fatal poisoning of livestock (19).

Description of *Clavibacter toxicus* sp. nov. *Clavibacter toxicus* (tox'i.cus. L. m. *toxicus*, poison). The description below is based on previously published data, the findings of this study, and our observations. *Clavibacter toxicus* cells are obligately aerobic, gram-positive, non-sporeforming, nonmotile, coryneform rods that are 0.6 to 0.75 by 1.5 μ m, including a capsule that is 0.08 to 0.14 μ m thick (1). Surface colonies on 523M agar are convex, smooth, entire, mucoid, and glistening, with light- to mid-yellow pigmentation. In old growth (>3 weeks) pale yellow variants are common and form streaks or convex protrusions, and they maintain their pigmentation in subsequent cultures. From cultured inoculum, single colonies take 5 to 6 days to appear, and from rehydrated anhydrobiotic material obtained from colonized ryegrass colonies take 8 to 14 days to appear. Colonies are often variable in size because the adhesive properties of the mucoid capsule make dispersal to single cells difficult, especially with cells obtained from solid media and colonized plants.

Cell walls contain 2,4-diaminobutyric acid and glycine but no diaminopimelic acid or ornithine (20). Acid is produced from galactose, mannose, and xylose but not from adonitol, dulcitol, erythritol, ethanol, inulin, mannitol, and melezitose. Acid production from xylose is variable. Acetate, glutamate, malonate, succinate, and tartrate are not utilized. The maximum concentration of NaCl tolerated is 1% (wt/vol). Strains do not grow at 37°C, and the optimum growth temperature is 26°C (17). Catalase is produced; oxidase, urease, tyrosinase, and acetoin are not produced. Nitrate is not reduced. Tween 80 and cotton seed oil are not lipolyzed. Unidentified growth factors are required for growth; biotin alone or in combination with nicotinic acid or calcium pantothenate is insufficient. All strains are lysed by the bacteriophage described by Riley and Gooden (NCPPB 3778) (21). The strains are slow growing, with a mean doubling time of 18 h in 523M broth at 25°C (twice the mean doubling time of the related organisms *Clavibacter tritici* and *Clavibacter rathayi*).

Allelic profiles differ by 20% between strains of *Clavibacter toxicus* and by at least 85% from strains of other *Clavibacter* spp. (24). Polyclonal antiserum raised in rabbits reacts strongly with all strains but cross-reacts at a reduced level with some other *Clavibacter* spp., *Arthrobacter ilicis*, and *Rhodococcus fascians* (20).

All of the strains tested adhere to some populations of the seed gall-forming nematode *Anguina funesta*, the nematode with which it is most commonly associated in infested ryegrass, and some other *Anguina* spp., such as *Anguina tritici*, with which it is not normally associated (22). The

bacterium colonizes seedheads and nematode galls in ryegrass (19) and occasionally the seedheads of other grasses at the same sites (2). It produces antibiotics that belong to the tunicamycin group (termed corynetoxins) in colonized plants (7); production of corynetoxins declines rapidly during isolation and subculture (16). Inoculation of host grasses has limited success without the vector nematode (17, 23).

The type strain is strain CS14 (= ICMP 9525 = NCPPB 3552). This strain is typical of *Clavibacter toxicus* in all of the tests shown in Table 2 and produces acid from xylose. Its DNA base composition is 67 mol% G+C. It was isolated in 1984 from ryegrass from a site in Murray Bridge, South Australia, where toxicity to grazing animals had been observed. The site was infested with the vector nematode, *Anguina funesta*.

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