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Leifsonia xyli-like bacteria are endophytes of grasses in eastern Australia

L. Mills[AE], *T. M. Leaman*[B], *S. M. Taghavi*[A], *L. Shackel*[A], *B. C. Dominiak*[C], *P. W. J. Taylor*[D], *M. Fegan*[B] *and D. S. Teakle*[A]

[A]Department of Microbiology and Parasitology, The University of Queensland, St Lucia, Queensland 4072, Australia.
[B]Cooperative Research Centre for Tropical Plant Protection, The University of Queensland, St Lucia, Queensland 4072, Australia.
[C]Bureau of Sugar Experiment Stations (BSES), Meiers Road, Indooroopilly, Queensland 4068, Australia; present address: NSW Agriculture, 161 Kite Street, Orange, NSW 2800, Australia
[D]Bureau of Sugar Experiment Stations (BSES), Meiers Road, Indooroopilly, Queensland 4068, Australia; present address: Molecular Plant Genetics and Germplasm Development Group, Department of Crop Production, The University of Melbourne, Parkville, Vic. 3010, Australia.
[E]Corresponding author; email: kahlo@smartchat.net.au

Abstract. Bacteria serologically related to *Leifsonia xyli* ssp. x*yli,* the causal bacterium of ratoon stunting disease (RSD) in sugarcane, were detected using the fluorescent antibody direct count on filter (FADCF) technique in grasses in eastern Australia. In a survey of 191 grass, sedge and bullrush samples comprising 53 plant species, 90 (47%) of the samples tested harboured bacteria which reacted positively with *L. xyli* ssp. *xyli* polyclonal antiserum. A total of 18 grass species was found to be naturally colonised with bacteria serologically related to and morphologically similar to *L. xyli* ssp. *xyli*. Grasses colonised by these *L. xyli*-like bacteria were present in areas both adjacent to, and removed from, sugarcane crops. When *L. xyli*-like bacteria from Rhodes grass (*Chloris gayana*) were inoculated into sugarcane, they multiplied at a lower rate than *L. xyli* ssp. *xyli*. *L. xyli*-like bacteria in Rhodes grass were isolated in axenic culture and exhibited growth rates, colony size and pigmentation similar to those of *L. xyli* ssp. *cynodontis*, a bacterial pathogen of *Cynodon dactylon* (couch grass). Further, using a polymerase chain reaction (PCR) test that could differentiate *L. xyli* spp. *xyli* from *L. xyli* spp. *cynodontis*, the *L. xyli*-like bacteria infecting Rhodes grass, couch grass and panic grass (*Panicum maximum*) generated a product of the same size as *L. xyli* spp. *cynodontis*. We conclude that *L. xyli* ssp. *cynodontis* or closely related bacteria are common endophytes of grasses in eastern Australia.

Additional keywords: ratoon stunting disease, serology, PCR, *Clavibacter.*

Introduction

Leifsonia xyli subspecies *xyli* (Davis *et al.*) Evtushenko, a coryneform bacterium (formerly of the genus *Clavibacter,* Evtushenko *et al.* 2000), infects the xylem of sugarcane (*Saccharum* interspecific hybrids) and causes ratoon stunting disease (RSD; Gillaspie and Teakle 1989) in susceptible sugarcane cultivars. Although sugarcane is the only known natural host of the bacterium, *L. xyli* ssp. *xyli* has been experimentally transmitted to 14 alternative hosts, namely elephant grass (*Pennisetum purpureum* Schum.) (Matsuoka 1971; Steindl 1957), bana grass (*P. purpureum* × *P. glaucum* (L.) R. Br.) (Steindl and Teakle 1974), maize (*Zea mays* L.) (Steindl 1961), panic grass (*Panicum maximum* Jacq.), para grass (*Brachiaria mutica* (Forsk.) Stapf), green summer grass (*B. milliformis* (Presl) Chase), barnyard grass (*Echinochloa colonum* (L.) Link), blady grass (*Imperata cylindrica* (L.) Beauv.), red Natal grass (*Rhynchelytrum repens* (Willd.) C.E. Hubbard), Parramatta grass (*Sporobolus capensis* (Willd.) Kunth), wild sorghum (*Sorghum verticilliflorum* (Steud.) Stapf) (Steindl 1957), Sudan grass (*Sorghum drummondii* (Steud.) Millsp. & Chase), Johnson grass (*Sorghum halepense* (L.) Pers.) (Liao and Chen 1981) and couch grass (*Cynodon dactylon* (L.) Pers.) (Liao and Chen 1981). These inoculated hosts remained symptomless.

A related bacterium, *L. xyli* ssp. *cynodontis*, occurs in, and causes stunting of, *Cynodon dactylon* (Liao and Chen 1981; Davis *et al.* 1984). This bacterium has been mechanically transmitted to Sudan grass (Liao and Chen 1981) and maize (Barbehenn and Purcell 1993). *L. xyli* sspp. *xyli* and *cynodontis* share some common cell surface antigens and both react with polyclonal antiserum produced against *L. xyli* ssp. *xyli*. They can be distinguished by their higher pathogenicity to their natural hosts, their different

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Table 1. Details of bacterial strains used in this study

Bacterial species	Strain designation	Source	Reactivity with antiserum[A]
Leifsonia xyli ssp. *xyli*	ACM 2272/L1A[T]	Sugarcane	+
L. xyli ssp. *cynodontis*	TB1A[B]	Couch grass	+
Clavibacter michiganensis ssp. *michiganensis*	ACM 4950[C]	Tomato	–
C. michiganensis ssp. *insidiosus*	ACM 4951	Lucerne	–
C. michiganensis ssp. *nebraskensis*	ACM 4952	Corn	–
Rathayibacter rathayi	ACM 4954	Cocksfoot grass	–
R. tritici	ACM 3998	Wheat	–
L. xyli-like	Clone 9	Rhodes grass	+
L. xyli-like	Clone 102	Rhodes grass	+

[A]An antiserum to *L. xyli* ssp. *xyli* ACM 2272/L1A was used in FADCF tests.
[B]Type strain.
[C]ACM = Australian Collection of Microorganisms, Department of Microbiology and Parasitology, The University of Queensland, St Lucia, Queensland 4072.

colony characteristics and growth rates on sugarcane (SC) agar (Davis *et al.* 1983) and by a polymerase chain reaction (PCR) test (Fegan *et al*. 1998).

Despite the implementation of RSD control measures, some Australian sugarcane farmers are unable to eradicate the disease from their crops. Dominiak *et al.* (1992) concluded that there might be previously undetected routes of infection, such as transmission from *L. xyli* ssp. *xyli*-infected grasses during harvesting of sugarcane fields. This paper reports a survey of grasses in eastern Australia to determine if they were naturally infected by *L. xyli* ssp. *xyli* or related bacteria.

Methods

Bacterial cultures

Reference and type strains of *L. xyli* and related bacteria were received from the Australian Collection of Microorganisms (ACM), The University of Queensland, Australia. In addition, *L. xyli*-like bacteria were isolated from Rhodes grass (*Chloris gayana*) in Queensland. Details of these bacterial strains are given in Table 1. *L. xyli* ssp. *xyli* and *L. xyli* ssp. *cynodontis* were maintained on modified sugarcane agar (MSC) (Croft *et al.* 1993). *C. michiganensis* and *Rathayibacter* spp. were maintained on 523M agar (Riley and Ophel 1992). All cultures were incubated at 28°C.

Production of polyclonal antiserum against L. xyli ssp. xyli

Antigen production

L. xyli ssp. *xyli* (ACM2271) isolated from sugarcane cultivar Q110 was suspended in 3 mL of sterile saline. The suspension was centrifuged at 15 000 rpm for 5 min and the pellet was washed and resuspended in 1.5 mL PBS (8 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4.12H_2O$, 0.2 g KCl, 1 L deionised water, pH 7.4). To the suspension, 1.5 mL of Freund's incomplete adjuvant was added and the mixture emulsified. A 1 mL volume of the emulsion was injected into the thigh muscle of a rabbit. This injection of freshly prepared bacterial suspension was repeated at fortnightly intervals for 3 months using a freshly prepared bacterial suspension each time.

Determination of antiserum titre

Approximately 3–5 mL of blood was collected at weekly intervals and stored overnight at 4°C to allow clotting. The serum was separated from the clot by centrifugation at 15 000 rpm for 5 min and the serum was subjected to two-fold dilutions in PBS. A drop of each serum dilution was transferred to a Petri dish and mixed with a drop of concentrated suspension of *L. xyli* ssp. *xyli* in PBS. Following a 1 h incubation at room temperature, the Petri dish was placed under a stereo microscope and each of the drops examined for agglutination. The highest dilution of serum at which agglutination occurred was considered to be the titre. After 12 weeks the rabbit was bled out when a titre greater than 1:1000 was reached.

Purification of immunoglobulin G (IgG)

Twenty mL of blood was collected 8 weeks following the initial injection and was centrifuged at 15 000 rpm for 5 min. The serum fraction was withdrawn, diluted 1:10 with sterile deionised water, and 50 mL of saturated ammonium sulfate (76.2 g / 100 mL deionised water) was added and the solution stirred for 45 min at room temperature. The precipitate was collected by centrifugation at 12 000 rpm for 10 min and dissolved in 10 mL of PBS. The suspension was dialysed twice in 1.5 L of half strength PBS overnight. IgG was separated from the other immunoglobulins by elution through a DEAE-cellulose column with PBS. Eluted fractions were collected in microfuge tubes and absorbances were determined spectrophotometrically at 280 nm. The approximate IgG concentration was calculated using the formula 1 mg mL^{-1} IgG = 1.4 absorbance units. Fractions with absorbances greater than 0.5 were retained.

Conjugation of IgG with fluorescein isothiocyanate (FITC)

The procedure followed was that of Davis and Dean (1984).

Specificity of the L. xyli ssp. xyli polyclonal antiserum using the fluorescent antibody direct count on filter (FADCF) technique

Bacterial isolates tested included *L. xyli* ssp. *xyli* (ACM2272/L1A – type strain), *L. xyli* ssp. *cynodontis* (TB1A – type strain), *C. michiganensis* sspp. *michiganensis* (ACM4950), *insidiosus* (ACM4951), and *nebraskensis* (ACM4952), *Rathayibacter rathayi* (ACM4954) and *R. tritici* (ACM3998). A loopful of culture of each bacterium to be tested was suspended in 500 µL of PBS and centrifuged at 15 000 rpm for 2 min. The pellet was resuspended in 200 µL of PBS, 5 µL of FITC-conjugated IgG was added and the suspension was incubated in the dark at 28°C for 30 min. The suspension was diluted in 2 mL of PBS and passed through a 0.2 µm filter membrane (Gelman Sciences, USA). Bacteria were deposited on the membrane and fluorescent-labelled bacteria were detected with PL Fluotar 100× objective on a Leitz Laborlux S microscope with a blue light illumination

Table 2. Grass species in south-east Queensland and northern New South Wales harbouring bacteria serologically related to *Leifsonia xyli* ssp. *xyli* as determined by the fluorescent antibody direct count on filter (FADCF) technique

Host	Locality	Number of colonised plants per number sampled
Brachiaria decumbens Stapf (signal grass)	Brisbane (Qld)	1/1
Chloris gayana Kunth (Rhodes grass)	Blue knob (NSW)	1/1
	Brisbane (Qld)	6/11
	Bundaberg (Qld)	7/11
	Chuwar (NSW)	1/1
	Gympie (Qld)	4/5
	Kholo Creek (NSW)	1/1
	Kilcoy (Qld)	1/1
	Kunghur (NSW)	1/1
	Mt. Glorious (Qld)	1/1
	North Maclean (Qld)	8/12
	Tumblegum (NSW)	1/1
	Wivenhoe (Qld)	2/2
Chloris virgata Sw. (feathertop Rhodes grass)	Delungra (NSW)	3/4
Cynodon dactylon (L.) Pers. (couch grass)	Brisbane (Qld)	3/6
	Chinderah (NSW)	3/3
Cynodon plectyostachyum Pilger (African star grass)	Bundaberg (Qld)	1/4
Echinochloa colonum (L.) Link (awnless barnyard grass)	Bundaberg (Qld)	3/6
Eleusine indica (L.) Gaertn. (crowsfoot grass)	Brisbane (Qld)	1/1
	Bundaberg (Qld)	4/5
Eragrostis sp. (love grass)	Bundaberg (Qld)	2/3
Eragrostis cilianensis (All.) Link ex Lutati (stink grass)	Bundaberg (Qld)	1/1
Imperata cylindrica (L.) Beauv. (blady grass)	Bundaberg (Qld)	3/4
	Chinderah (NSW)	1/1
	Cudgen (NSW)	1/1
Melinus minutiflora P. Beauv. (molasses grass)	Cudgen (NSW)	1/1
	Tweed Valley (NSW)	1/1
Panicum maximum Jacq. var. *trichoglume* Eyles ex Robyns (panic grass)	Cudgen (NSW)	6/6
Paspalum dilatatum Poir. (paspalum)	Brisbane (Qld)	2/4
	Bundaberg (Qld)	3/4
Paspalum urvillei Steud. (Vasey grass)	Brisbane (Qld)	3/3
	Mt. Glorious (Qld)	1/1
Rhynchelytrum repens (Willd.) C.E. Hubb. (red Natal grass)	Brisbane (Qld)	1/1
Setaria gracilis Beauv. (pale pigeon grass)	Bundaberg (Qld)	1/2
Sorghum verticilliflorum (Steud.) Stapf (wild sorghum)	Brisbane (Qld)	1/2
	Tweed Valley (NSW)	1/1
Sporobolus caroli Mez (fairy grass)	Gympie (Qld)	2/2

(12 filter block) (Ernst Leitz Ltd., Germany). A strong uniform fluorescence of cells was deemed a positive reaction with the antiserum.

Serological detection of Leifsonia xyli-like bacteria in plants

Extraction of bacteria from plants

Grasses, sedges and a bullrush from locations in south-east Queensland and north-east New South Wales were sampled. Plants were removed from the soil with their roots intact. Soil and debris were removed from stalks by washing under running water. Stalks were cut into approximately 5 mm pieces, placed in a 25 mL bottle and immersed in PBS/Azide (500 mL PBS / 0.1 g NaN_3) for 2 h. A 1.5 mL portion of the suspension was transferred to a microfuge tube and centrifuged at 12 000 rpm for 5 min. The pellet was resuspended in 200 µL of PBS.

Inoculation of sugarcane setts

Sources of inoculum

Rhodes grass (*Chloris gayana*) clones 9 and 102 were maintained in a glasshouse at about 25°C and were watered regularly. Each pot was elevated and isolated to prevent cross contamination during irrigation and manipulations. The grasses were shown by FADCF to be infected with *L. xyli*-like bacteria. Stalks (approximately 200 g) of each clone were cut aseptically into 5–10 mm-long pieces. The cut pieces were added to 500 mL of sterile deionised water and the suspension was kept

at room temperature for 2 h. The liquid portion of the suspension was decanted and retained.

Sugarcane cultivar Q110 known to be infected with *L. xyli* ssp. *xyli* was maintained at the BSES Pathology Farm, Brisbane. Five stalks were pressed to express the juice and the extract was diluted 1:1 with sterile deionised water. FADCF was used to determine the presence of *L. xyli* ssp. *xyli* in the extract. The initial inoculum concentration of *L. xyli* ssp. *xyli* and *L. xyli*-like bacteria was approximately 10^5 cells/ mL.

Source of soil and RSD-free sugarcane Twenty stalks of apparently healthy sugarcane variety Q110, maintained at the BSES Pathology Farm, Brisbane, were steam treated at 52°C for 4–5 h (a treatment designed to cure plants of *L. xyli* ssp. *xyli* infection; Gillaspie and Teakle 1989). Single node setts were cut using alcohol-sterilised cutters. Fibrovascular sap of heated sugarcane stalks was extracted under positive pressure (Croft and Witherspoon 1982) and found by FADCF to be free of *L. xyli* ssp. *xyli*. Approximately 100 kg of soil from the BSES Pathology Farm was crudely sieved to remove large pieces of organic matter and steam treated at 52°C for 3 h.

Inoculation of sugarcane uprights

Pots (190 × 210 mm) were washed, rinsed with 70% ethyl alcohol and filled with steamed soil to a depth of approximately 170 mm. Both cut ends of 20 healthy Q110 single-node setts were sprayed until saturated with the appropriate inoculum and the setts incubated for 1 h. The setts were then planted horizontally in the pots with the nodal bud uppermost, with four pots containing five single node setts per treatment. A control treatment was also included in which the setts were left untreated. The pots were maintained in a glasshouse at about 25°C. Two stalks from each pot were sampled at 5 and 8 months following inoculation. Fibrovascular extract was extracted via positive pressure from these stalks and the FADCF technique was used to detect *L. xyli* ssp. *xyli* and *L. xyli*-like bacteria. The extract was deemed positive if one or more bacteria per field were observed in ten microscope fields. The remaining fifth stalk from each pot was sliced longitudinally after 8 months to determine if vascular discoloration had occurred.

In vitro isolation of L. xyli-like bacteria

Rhodes grass cultivars 9 and 102 which harboured *L. xyli*-like bacteria were maintained in a glasshouse at about 25°C. Stalks were washed in tap water, blotted dry and cut into pieces 50–60 mm in length. The stalk pieces were immersed in 5% (w/v) sodium hypochlorite and 10% (v/v) Tween 20 for 5 min and rinsed in sterile deionised water. Approximately 10 mm was cut aseptically off a piece from one end and discarded. The sap was expressed from the remaining tissue directly onto MSC agar plates and incubated at 28°C.

PCR for the differentiation of L. xyli ssp. xyli and ssp. cynodontis

A PCR assay used to distinguish *L. xyli* ssp. *xyli* from ssp. *cynodontis* (Fegan *et al.* 1998) was used to amplify DNA from *L. xyli*-like isolates obtained from Rhodes grass. The PCR reaction contained three primers: a conserved forward primer which would anneal to either ssp. *xyli* or ssp. *cynodontis*, and two reverse primers which were specific for either ssp. *xyli* or ssp. *cynodontis.* The type strains for *L. xyli* sspp. *xyli* (ACM2272/L1A) and *cynodontis* (TB1A) were used as positive controls. The PCR test was also used in conjunction with the FADCF technique to test for the presence of *L. xyli* ssp. *cynodontis* in a total of 17 extracts obtained from eight grass species.

Results

Specificity of L. xyli ssp. xyli polyclonal antiserum

Polyclonal antiserum with a homologous agglutination titre of 1:1430 against *L. xyli* ssp. *xyli* was produced 3 months after the first injection. Using the FADCF technique, the antiserum reacted with *L. xyli* ssp. *xyli* cells and exhibited cross-reactivity with *L. xyli* ssp. *cynodontis* cells. The antiserum did not react using FADCF with cells of *C. michiganensis* subspp. *michiganensis*, *insidiosus* and *nebraskensis*, *Rathayibacter tritici* or *R. rathayi*.

Presence and geographical location of plants harbouring L. xyli ssp. xyli

In a survey of 191 grass, sedge and bullrush samples obtained from 53 plant species, 90 of the samples harboured bacteria that reacted positively using FADCF with *L. xyli* ssp. *xyli* polyclonal antiserum. A total of 18 grass species of the family Poaceae was found to be naturally colonised with bacteria serologically related to, and morphologically similar to, *L. xyli* ssp. *xyli* . The colonised grass species and the localities from which they were obtained are shown in Table 2.

Grass, bullrush and sedge species which did not harbour a natural population of *L. xyli*-like bacteria were *Andropogon gerardii* Vitm. (big bluestem), *Axonopus compressus* P. Beauv. (broadleaf carpet grass), *Bothriochloa ewartiana* (Domin) C.E. Hubb. (desert blue grass), *Bothriochloa insculpta* (Hochst. ex A. Rich.) A. Camus cv. Bisset (creeping blue grass), *Brachiaria miliiformis* (Presl) Chase (green summer grass), *Bromus diandrus* Roth (great brome), *Capillipedium spicigerum* S.T. Blake (scented top), *Cenchrus ciliaris* L. (buffel grass), *Cyperus brevifolius* (Rottb.) Haask. (Mullumbimby couch), *C. difformis* L. (rice weed), *C. rotundus* L. (nut grass), *Dicanthium aristatum* (Poiret) C.E. Hubb. (Angelton grass), *Digitaria ciliaris* (Retz.) Koeler (summer grass), *D. parviflora* (R. Br.) Hughes (small flower finger grass), *D. sanguinalis* (L.) Scop. (crab grass), *Hemarthria altissima* (Poir) Stapf & C.E. Hubb. (limpo grass), *Heteropogon contortus* (L.) P. Beauv. ex Roem. and Schult. (black speargrass), *Panicum antidotale* Retz. (blue panic grass), *Paspalum nicorae* Parodi (Brunswick grass), *P. notatum* Hugge cv. Competidor (Bahia grass), *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu), *P. glaucum* (L.) Pers. (pearl millet), *Setaria anceps* Stapf (setaria), *Sorghastrum nutans* (L.) Nash cv. Lometa (yellow Indian grass), *Sorghum halepense* (L.) Pers. (Johnson grass), *Sporobolus pyramidalis* P. Beauv. var. *pyramidalis* (giant rats tail grass), *Typha* sp. (bullrush), *Urochloa mosambicensis* (Hack.) Dandy (sabi grass), *U. panicoides* P. Beauv. var. *panicoides* (liverweed grass) and *Vetiveria zizanioides* (L.) Nash (Vetiver grass). It was noted that the size of the pellet obtained from diffusates of these plants was significantly smaller than that of Rhodes grass and thus the population of *L. xyli*-like bacteria, if present, may have been below the level of detection of the FADCF technique.

Infection in sugarcane

Five months after inoculation of sugarcane setts with *L. xyli* ssp. *xyli* from sugarcane, fluorescent cells resembling those of this bacterium were detected in fibrovascular extracts of all eight stalks tested. At this time, no fluorescent

Table 3. The number of stalks infected, and the average concentration of fluorescent *Leifsonia xyli*-like bacteria in extracts, 8 months post-inoculation of sugarcane Q110 with *L. xyli* ssp. *xyli* or *L. xyli*-like bacteria.

	No. stalks infected	Av. conc. bacteria/mL	No. stalks infected	Av. conc. bacteria/mL
Sugarcane juice extract	8/8	3.4×10^8	8/8	4.7×10^8
Rhodes grass clone 9 extract	0/8	0	4/8	1.2×10^6
Rhodes grass clone 102 extract	0/8	0	3/8	0.9×10^6
Control — untreated	0/8	0	0/8	0

[A] The extracts from sugarcane (containing *L. xyli* ssp. *xyli*) and Rhodes grass (*L. xyli*-like bacteria) each contained approx. 10^5 cells/mL

bacteria were detected in sugarcane inoculated with the *L. xyli*-like bacteria from Rhodes grass or in the uninoculated controls (Table 3).

Eight months after inoculation with *L. xyli* ssp. *xyli*, fluorescent cells resembling those of this bacterium were again detected in 8/8 stalks of sugarcane examined (100% colonisation). However, fluorescent bacteria were also seen in 4/8 and 3/8 sugarcane stalks inoculated with *L. xyli*-like bacteria from Rhodes grass clone 9 and clone 102, respectively. The fluorescent bacteria were more numerous (approx. 10^8 cells/mL) in the extracts of infected sugarcane inoculated with *L. xyli* ssp. *xyli* than those inoculated, with the *L. xyli*-like bacteria from Rhodes grass (approx. 10^6 cells/mL; Table 3).

The typical RSD symptoms of a red/orange discoloration of the vascular bundles in nodes of mature stalks, were not apparent when sugarcane inoculated with either *L. xyli* ssp. *xyli* or the *L. xyli*-like bacteria from Rhodes grass was examined after 8 months.

In vitro isolation of L. xyli-like bacteria

Six *L. xyli*-like isolates were obtained in pure culture on MSC agar from six Rhodes grass clones of cultivars Pioneer, Topcut or Finecut. The bacteria grew aerobically on MSC agar, producing colonies of approximately 0.5–1 mm diameter in 3–5 days. Colonies were circular with entire margins, were convex and developed yellow pigmentation. Isolation of *L. xyli*-like bacteria from other grasses was unsuccessful.

Identification of L. xyli-like bacteria using PCR

The six *L. xyli*-like strains obtained from Rhodes grass produced a 446 bp amplification product specific for *L. xyli* ssp. *cynodontis* (not shown). *L. xyli*-like bacteria were also detected using both PCR and FADCF in the fibrovascular extract of nine samples of Rhodes grass from which isolates were not obtained and in xylem exudate from *Cynodon dactylon* (couch grass) and *Panicum maximum* (panic grass).

Discussion

The only reported natural host of *L. xyli* ssp. *xyli* is sugarcane and the bacterium can be transmitted from diseased to healthy stalks on harvesting equipment (Hughes and Steindl 1955; Taylor *et al.* 1988). Despite its narrow host range in nature, the RSD bacterium has been shown to infect 14 grass species under experimental conditions (Steindl 1957; Liao and Chen 1981). Dominiak *et al*. (1992) suggested that grasses are undetected reservoirs of the RSD bacterium and that the bacterium might be transmitted to sugarcane during harvesting. To investigate this possibility we surveyed selected plants in subtropical eastern Australia.

The survey of grasses, sedges and a bullrush in south-east Queensland and northern New South Wales using FADCF revealed that, out of 191 samples tested, 90 were positive for bacteria which were serologically and morphologically related to *L. xyli* ssp. *xyli*. A total of 18 grass species of the family Poaceae harboured natural populations of *L. xyli*-like bacteria (Table 2). These grasses were widely distributed in subcoastal areas. Grasses colonised by *L. xyli*-like bacteria were present in areas both near to, and remote from, commercial sugarcane cultivation areas. For instance, infected *Eleusine indica* and *Paspalum dilatatum* plants were found in the Brisbane area, in addition to growing adjacent to sugarcane fields in Bundaberg. Since *L. xyli*-like infected grasses were not exclusively found in sugarcane regions, there was no evidence that the colonised grasses were always being infected from sugarcane.

At 5 months post-inoculation, infectivity studies demonstrated that *L. xyli*-like bacteria from Rhodes grass were not detected in sugarcane cultivar Q110, and they had increased to only approximately 10^6 cells/mL of extract at 8 months. Since the limit of sensitivity of the FADCF technique is 10^4–10^5 cells/mL, it is assumed that after 5 months the population of *L. xyli*-like bacteria in sugarcane was below the threshold of detection. In contrast, *L. xyli* ssp. *xyli* had multiplied in sugarcane to approximately 10^8 cells/mL of extract after 5 months. In addition, while *L. xyli* ssp. *xyli* colonised 100% of inoculated sugarcane setts, *L. xyli*-like bacteria from Rhodes grass clones 9 and 102 were detected in only 50% and 37.5% of setts, respectively.

While *L. xyli* ssp. *xyli* is able to infect non-natural hosts, these infections occur at a lower efficiency and give rise to lower bacterial yields when compared to infections of natural hosts. Davis *et al.* (1983) observed that *L. xyli* ssp. *xyli* did not readily multiply within *Cynodon dactylon* and Liao and Chen (1981) reported that *L. xyli* ssp. *cynodontis* colonised only 22% of Sudan grass-sorghum hybrids, while *L. xyli* ssp.

xyli infected 92% of the plants. It can thus be inferred from this that the *L. xyli*-like bacteria isolated from *Chloris gayana* are probably not *L. xyli* ssp. *xyli* based on their inability to colonise, and propagate within, sugarcane vascular tissue to the corresponding level exhibited by *L. xyli* ssp. *xyli*.

While the presence of vascular discoloration in mature nodes of sugarcane has been used to diagnose *L. xyli* ssp. *xyli* infection (Hughes and Steindl 1955; Steindl and Teakle 1974; Taylor *et al.* 1988; Gillaspie and Teakle, 1989), no discoloration or other symptoms of disease were observed in this study. Others have reported asymptomatic infection of sugarcane (Steindl 1961), and discoloration is known to vary among sugarcane clones (Gillaspie and Teakle 1989). It was expected that the susceptible Q110 cultivar used in this study would exhibit symptoms of disease. However, RSD is notably a disease that is observed during adverse growing conditions in the field. These conditions may not have been represented under the experimental conditions used in this study. Thus the lack of observable symptoms precludes the determination of the pathogenic role of *L. xyli*-like bacteria in RSD of sugarcane.

Davis *et al.* (1988*a*) reported symptomatic colonisation of sugarcane with bacterial yields of 8.5×10^8 cells/mL from a mature internode, which is similar to the yields achieved in this study (4.7×10^8 cells/mL). The level of colonisation within sugarcane cultivars has also been shown to be an indicator of yield loss (Koike *et al.* 1982; Davis *et al.* 1988*b*). Therefore, the failure of the *L. xyli*-like bacteria from Rhodes grass to multiply to the level seen in *L. xyli* ssp. *xyli*-infected sugarcane cultivar Q110 suggests the *L. xyli*-like bacteria may be less pathogenic to sugarcane than *L. xyli* ssp. *xyli*.

Isolates of *L. xyli*-like bacteria from Rhodes grass in axenic culture exhibited growth rates and colony size and pigmentation similar to *L. xyli* ssp. *cynodontis*. *L. xyli*-like isolates from Rhodes grass generated a PCR product the same size as *L. xyli* ssp. *cynodontis*, using a PCR test that can differentiate *L. xyli* ssp. *xyli* from ssp. *cynodontis* (Fegan *et al.* 1998). Due to the difficulties associated with isolating these fastidious, slow growing bacteria from plants, isolates were not obtained from grasses other than Rhodes grass. It was observed that Rhodes grass harboured a larger population of *L. xyli*-like bacteria than the other grasses examined and this may have contributed to the relative ease of isolation of these bacteria.

As isolates could be obtained only from Rhodes grass, extracts of other grasses were subjected to PCR tests. *L. xyli*-like bacteria were detected by PCR (producing a product the same size as that produced by *L. xyli* ssp. *cynodontis*) in Rhodes grass (*Chloris gayana*), couch grass (*Cynodon dactylon*) and panic grass (*Panicum maximum*). The observation that Rhodes grass harboured a larger population of *L. xyli*-like bacteria than other grasses may have also contributed to the low number of grasses that tested positive by PCR. The number of bacteria present in other grasses may have been below the limit of detection of the PCR test used (10^4 cells/mL; Fegan *et al*. 1998).

We have shown that *L. xyli*-like bacteria naturally infected some plants of Rhodes grass (*Chloris gayana*), couch grass (*Cynodon dactylon*) and panic grass (*Panicum maximum*) in eastern Australia. *L. xyli*-like bacteria were present, sometimes in low numbers, in 15 other grasses in eastern Australia. The significance of these endophytes in the health and productivity of their host grasses in Australia remains to be determined.

It is possible that these *L. xyli*-like bacteria could be mechanically transmitted from their grass hosts to sugarcane during harvesting operations. If the results of our inoculation test with the Rhodes grass isolates are applicable to isolates from other grasses, they are unlikely to cause significant disease in sugarcane. However, their cross-reactivity with *L. xyli* ssp. *xyli* antiserum in routine screening for RSD in sugarcane could conceivably result in false positive diagnoses.

The exact identity of the *L. xyli*-like bacteria from Rhodes grass and other grasses in eastern Australia has not yet been determined. Although at least some resemble *L. xyli* ssp. *cynodontis* in having a faster growth rate and a yellower colony type on MSC agar than *L. xyli* ssp. *xyli*, further phenotypic and pathogenicity tests are needed to support evidence of similarity with *L. xyli* ssp. *cynodontis* provided by the PCR tests.

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