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# Investigation of *Leifsonia xyli* subsp. *xyli* in insects

**Investigação de Leifsonia xyli subsp. xyli em insetos**

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**Abstract**
*Leifsonia xyli* subsp. *xyli*, the causal agent of ratoon stunting, is an endophytic bacteria that colonize sugarcane xylem vessels. It is generally accepted that sugarcane is the only natural host of ratoon stunting. The objective of this work was to investigate the presence of bacteria in insects associated with sugarcane in an experimental area cultivated with four varieties. The plants were artificially inoculated with bacteria, the samples of insects were collected from the sugarcane crop. Specific PCR was used with DNA extracted from different insect parts (head, abdomen, or whole-body). The DNA of the bacterium was not detected in those investigated insects, confirming that the host range of the bacterium is restricted to *Saccharum* spp.
**Keywords:** *Saccharum*; DNA; Sugarcane ratoon stunting.

**Resumo**
*Leifsonia xyli* subsp. *xyli*, o agente causal do nanismo de ratos, é uma bactéria endofítica que coloniza os vasos do xilema da cana-de-açúcar. É geralmente aceito que a cana-de-açúcar é o único hospedeiro natural do raquitismo da soca. O objetivo deste trabalho foi investigar a presença de bactérias em insetos associados à cana-de-açúcar em uma área experimental cultivada com quatro variedades. As plantas foram inoculadas artificialmente com bactérias, as amostras de insetos foram coletadas da cultura da cana-de-açúcar. PCR específico foi usado com DNA extraído de diferentes partes do inseto (cabeça, abdômen ou corpo inteiro). O DNA da bactéria não foi detectado nos insetos investigados, confirmando que a gama de hospedeiros da bactéria é restrita a *Saccharum* spp.
**Palavras-chave:** *Saccharum*; DNA; Raquitismo da soqueira.

**Resumen**
*Leifsonia xyli* subesp. *xyli*, el agente causal del retraso del crecimiento en ratas, es una bacteria endofítica que coloniza los vasos del xilema de la caña de azúcar. En general, se acepta que la caña de azúcar es el único huésped natural del retraso en el crecimiento de los retoños. El objetivo de este trabajo fue investigar la presencia de bacterias en insectos asociados a la caña de azúcar en un área experimental cultivada con cuatro variedades. Las plantas fueron inoculadas artificialmente con bacterias, las muestras de insectos fueron recolectadas del cultivo de caña de azúcar. Se utilizó PCR específica con ADN extraído de diferentes partes del insecto (cabeza, abdomen o cuerpo entero). El ADN de la bacteria no se detectó en los insectos investigados, lo que confirma que el rango de huéspedes de la bacteria está restringido a *Saccharum* spp.
**Palabras clave:** *Saccharum*; ADN; Raquitismo de retoño.

## 1. Introduction

Sugarcane Ratoon Stunting Disease (RSD) is a bacteria that is critical in shaping plant health for abiotic and biotic stress tolerance (Carneiro et al., 2021). The *Leifsonia* genus includes pathogenic and beneficial bacteria, but limited research is available comparatively across species regarding their association with plants (Nordstedt et al., 2021). The genus *Leifsonia* comprises one species and 14 subspecies isolated from very diverse environments (Reddy et al., 2003), such as the Himalayan lagoon (Reddy et al., 2008; Singh et al., 2020). *Leifsonia xyli* is the only pathogenic species described so far and comprises two subspecies: *Leifsonia xyli* subsp. *cynodontis*, a pathogen of Bermuda grass (*Cynodon dactylis*) and *Leifsonia xyli* subsp. *xyli* (Lxx), which causes Ratoon Stunting Disease (RSD) in sugarcane (Davis et al., 1984; Cursi et al., 2022). Sugarcane is generally accepted as its only natural host (Zavaglia et al., 2016).

Lxx can be easily found worldwide where sugarcane is commercially grown. In Brazil, research has detected the pathogen in 23-67% of the field sampled (Ponte et al., 2010; Urashima; Marchetti, 2013). Surprisingly, however, few studies of natural alternative hosts of Lxx are in literature (Mills et al., 2001; Zavaglia et al., 2016; de Castro Moretti, 2018). Unlike other xylem-restricted bacteria, such as *Xylella fastidiosa* and *Xanthomonas albilineans*, which have a wide range of host plants (Tokeshi; Rago, 2005; Clavijo-Coppens et al., 2021), to date, there are no reports of alternative hosts for Lxx (Zavaglia et al., 2016; Zhang et al., 2021). However, there is also no systematic study conducted with this focus. In *Xylella*, insects can acquire the bacteria by feeding on infected plants and transmitting it to a healthy plant after a period that varies between 1 and 2 hours. Adult insects can transmit for the rest of their lives, and nymphs only until the next instar of development.

The re-establishment of a new sugarcane field occurs annually in 15 to 20% of the cultivated area of the Brazilian sugar industry, a territory that covered 10.1 million hectares in Brazil in 2018. RSD, caused by *Leifsonia xyli* subsp. *xyli* (Lxx) is a systemic disease transmitted through contaminated propagation materials. There are reports of high levels of Lxx infection in all cultivars with an average of 40.2%, in the study period, with an increasing tendency of contamination by Lxx. In addition, all cultivars showed high susceptibility to RSD, demonstrating the potential damage to the Brazilian sugar-energy sector and the need for the sector to adopt stricter sanitation practices for the formation of the sugarcane nursery (Urashima et al., 2020).

Diagnosis of the disease is based on detection of the bacteria by numerous techniques, including phase contrast microscopy (Steindl, 1976; Abdalla, 2020), serology (Gillaspie, 1978; Rott et al. 2018), or molecular methods that are highly sensitive and have been developed for the detection of *Leifsonia xyli* subsp. *xyli*, including Polymerase Chain Reaction (PCR) (Fegan et al., 1998; Pan et al., 1998; Taylor et al., 2003; Wu et al., 2018) and Nested-PCR (Pelosi et al., 2013; Dias et al., 2020).

Because RSD occurs worldwide and is a significant cause of sugarcane productivity, a better understanding of the processes related to Lxx can help develop tools and technologies to produce sugarcane varieties of RSD-resistant sugars through conventional and/or molecular breeding (Zhu et al., 2021). Additional investigations of alternative hosts of Lxx are essential to better understand its biology and its dispersion in sugarcane fields. The objective of this work was to investigate whether *Leifsonia xyli* subsp. *xyli* may have essential sugarcane insects as hosts.

## 2. Methodology

### *2.1 Insect collection*

The insects were obtained at different periods (Table 1) in an experimental sugarcane plantation implanted in the Rural Campus – experimental farm of the Federal University of Sergipe (lat. 10°55'26"S, long. 37 °11'57"O).

**Table 1.** Insect species collected in sugarcane inoculated with *Leifsonia xyli*.

| Species | Family | Season |
|---|---|---|
| *Diatraea flavipennella* | Lepidoptera: Crambidae | Summer / Winter |
| *Diatraea saccharalis* | Lepidoptera: Crambidae | Summer |
| *Mahanarva fimbriolata* | Hemiptera: Cercopidae | Summer/Winter |
| *Mahanarva posticata* | Hemiptera: Cercopidae | Summer/Winter |
| *Heterotermes tenuis* | Isoptera: Rhinotermitidae | Summer |

Source: Authors.

The area has 1,500 $m^2$ and contains more than 100 plants distributed in 4 varieties: Co 997, RB867515, RB92579, and RB951541. These plants were artificially inoculated by cutting the plants using an instrument previously immersed in Lxx inoculum suspension two years before the insect collection. The inoculum was prepared by subculture and culturing the isolated bacteria in the S8 liquid culture medium described by Davis et al. (1984). Inoculation was performed by cutting the plants at 180 days of age using a cutting instrument previously immersed in the inoculum suspension. Approximately 10 ml of the same suspension was applied to the exposed surface of the cut plants was performed to ensure inoculation. Through PCR assays, it was possible to confirm the identity of the isolate as being the bacterium *L. xyli* subsp. *xyli*, the causal agent of the disease ratoon rickets (RSD) (Urashima, 2010).

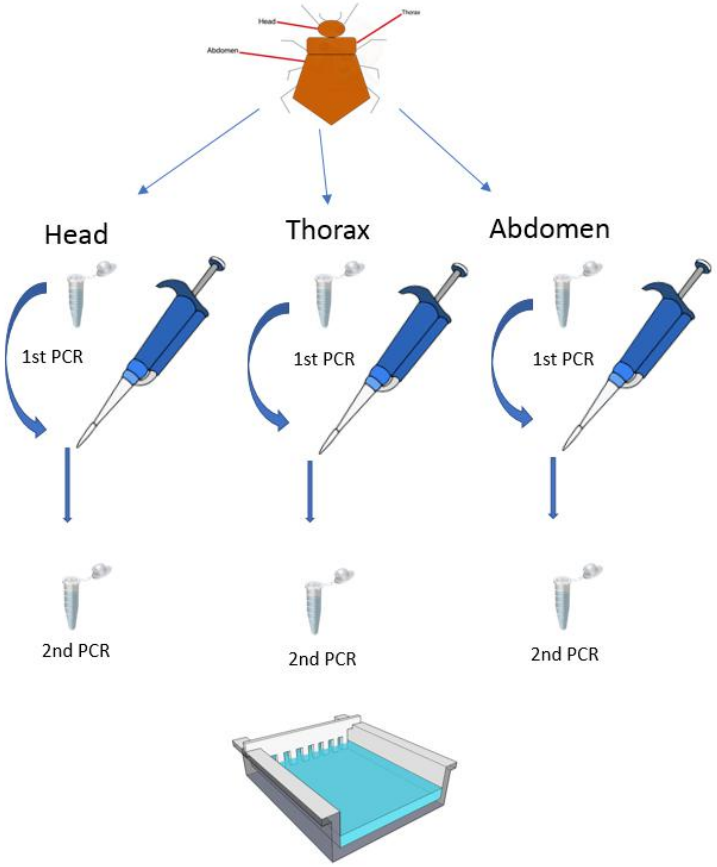
The choice of insects was based on the sugarcane pest identification manual (Garcia, 2013), which presents a list of the country's main pests for the sugarcane and ethanol sector (2013). The insects collected were identified and immediately stored in liquid nitrogen (Table 1).

The insects were used for DNA extraction as guided by the EMBRAPA 68 technical bulletin for sample preservation and insect DNA extraction (De Souza et al., 2013).

### *2.2 DNA extraction and optimizations*

The insects were kept in liquid nitrogen until the use and were macerated in a mortar with a pestle. Subsequently, the DNA was extracted according to Carvalho (2016). The method was developed for extracting mycoplasmas from insects (Vega et al., 1993). The following insect parts were used for DNA extraction: head, abdomen, and insect whole body (Figure 1).

**Figure 1.** Insect parts used for DNA extraction.




Source: Authors.

Initially, the samples were transferred to 1.5 mL microtubes, frozen in liquid nitrogen, and macerated with the aid of a conical-tipped pestle. Groups of one to five insects were macerated in 400 mL of extraction buffer (100 mM Tris; 50 mM EDTA; 500 mM NaCl, pH 8.0), added with 2 mL of b-mercaptoethanol and sodium dodecyl sulfate, at a final concentration of 1%, followed by centrifugation at 2,000 rpm for 10 minutes. The supernatant was collected and heated for five minutes at 65°C. Nucleic acids were extracted with TE-saturated chloroform/isoamyl alcohol/phenol (100 mM Tris; 50 mM EDTA; 500 mM NaCl, pH 8.0). The DNA was precipitated with 2.5 volumes of absolute ice-cold ethanol for 30 minutes at -80°C, then washed with 70% ethanol. After drying, the DNA was resuspended in 100 mL of 6X SSC (0.9 M sodium chloride, 0.09 M sodium citrate, pH 7.0).

### *2.3 Detection of Leifsonia xyli subsp. xyli in insects*

DNA was extracted from insect samples for amplification of genomic sequences, using the following pairs of primers: LX23SF (ACCTCCTTTCTAAGGAGC), LX23SR(TGAATTGATCGGCTCACC), and Cxx1 (CCGAAGTGAGCAGATTGACC), Cxx2 (ACCCTGTGTTGTTTTCAACG).

The samples were identified according to the insect species, and the body part was sampled for analysis (Table 2).

**Table 2.** Identification, species, and part of the insect used for DNA extraction for the detection of *Leifsonia xyli*.

| Identification | Species | Part of insect |
|---|---|---|
| 1 | *Diatraea flavipennella* | whole |
| 2 | *Diatraea saccharalis* | whole |
| 3 | *Mahanarva fimbriolata* | head |
| 4 | *Mahanarva fimbriolata* | Abdomen |
| 5 | *Mahanarva posticata* | head |
| 6 | *Mahanarva posticata* | Abdomen |
| 7 | *Heterotermes tenuis* | whole |
| 8 | *Mahanarva posticata* – nymph stage | head |
| 9 | *Mahanarva posticata* – nymph stage | Abdomen |

Source: Authors.

In Table 2 the species of the insects and part used are presented. The preparation of 25 µL of the mix for the PCR reaction was carried out, and the assays of the reactions were occurred according to the work described by Pan et al. (1998). Two microliters of insect DNA were added in microtubes containing the amplification buffer (1X), 200 µM of each dNTP, 0.2 µM of primers LX23SF (5'ACCTCCTTTCTAAGGAGC-3') and LX23SR (5'-TGAATTGATCGGCTCACC-3'), drawn from the 23S region of ribosomal RNA, and 1 U of Taq DNA polymerase. The reaction was carried out in a PTC 100 thermocycler (MJ Research®, USA) using the following cycles: an initial cycle at 94°C for 5 minutes, 40 denaturation cycles at 95°C for 30 seconds, annealing at 57°C for 1 minute, and extension at 72°C for 1.5 minutes, and a final extension cycle at 72°C for 3 minutes.

The amplified products were separated by electrophoresis on 1.0% agarose gel in 0.5X TBE buffer (50 mM tris-base, 50 mM boric acid and 1 mM EDTA - pH 8.5) for 3 hours at 5 V/cm. Under ultraviolet light, the gel was stained and photographed in a MasterR VDS Image Documenter (Pharmacia Biotech®, San Francisco, CA, USA). The sizes of the generated fragments were estimated based on comparisons with a 100 bp DNA Ladder molecular marker (Promega Corporation®, Madison, WI, USA).

**Table 3.** PCR reaction used for detection of *Leifsonia xyli* in insects.

| PCR reaction | Volume |
|---|---|
| $H_2O$ | 13.95 µL |
| Buffer enzyme 10x | 2 µL (1x) |
| $MgCl_2$ (50 mM) | 2 µL (3.0 mM) |
| dNTPs (1.25 mM) | 4 µL (200 mM) |
| Primer R (25 pmoles $mL^{-1}$) | 0.4 µL (10 pmoles) |
| Primes F (25 pmoles $mL^{-1}$) | 0.4 µL (10 pmoles) |
| Taq (5 U $mL^{-1}$) | 0.25 µL (1.25 U) |
| DNA (insect) | 2 µL |
| Final Volume | 25 µL |

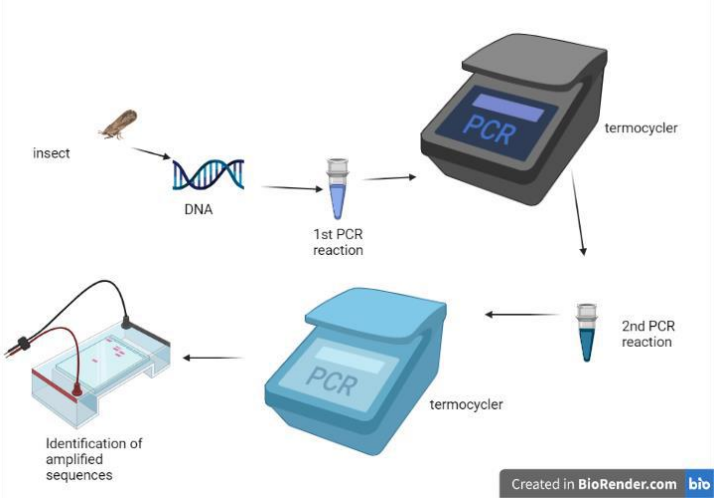
Source: Authors.

The PTJ-100-60 thermocycler (MJ Research®, Inc. Watertown, MA, USA) was used for DNA amplification. In the amplification of the Lxx DNA with the different primers, different amplification programs were also used. For primers Cxx1 (CCGAAGTGAGCAGATTGACC), Cxx2 (ACCCTGTGTTGTTTTCAACG), the samples were initially subjected to initial denaturation at 95°C for one minute, followed by 35 cycles with denaturation at the same temperature for 30 seconds, annealing at 55°C and 61° C (internal) for 30 seconds and extension at 72°C for 45 seconds, followed by a final extension of three minutes at 72°C.

***2.4 Nested-PCR***

The presence of the bacteria was investigated through the amplification of a fragment of the viral genome in Nested-PCR. Primers Cxx1 (CCGAAGTGAGCAGATTGACC), Cxx2 (ACCCTGTGTTGTTTTCAACG) and LX23SF (5'ACCTCCTTTCTAAGGAGC-3') and LX23SR (5'-TGAATTGATCGGCTCACC-3') were used. In Nested-PCR, the primers used in the first round of amplification were replaced by others of the same sequence so that the DNA amplified in conventional PCR becomes the target for the second pair of primers, increasing the sensitivity and specificity of PCR (Figure 2).

Figure 2. PCR and Nested-PCR test for *Leifsonia xyli* (Lxx) in DNA from insects in sugarcane.




Source: Authors.

In the Figure 2 are presented the sequences to proceeds the essay. The amplification conditions were the same as for the PCR reaction: initial heating at 95ºC for 5 minutes, 35 cycles of initial denaturation at 94ºC for the 30s, annealing at 58ºC for 30s, and extension at 72ºC for 35s; and a final extension at 72°C/10 min. Ten microliters of the PCR reaction were submitted to electrophoresis in a 1.5% agarose gel in TAE buffer, with an electrical voltage of 80 Volts, for 40min. The PCR products were visualized in a UV transilluminator (Loccus®) and photo documented.

Two-step nested PCR was performed using the same reaction mixture and cycling conditions as reported for conventional PCR.

## 3. Results and Discussion

***3.1 Conventional PCR with different primers for detection of Leifsonia xyli subsp. xyli with different hosts.***

Among the analyzed samples, there were no amplifications. For samples 7, 8, and 9, there were artifacts, later with the same DNA as in the previous analysis, a NESTED-PCR was performed. In the second reaction, NESTED-PCR, no amplifications were seen. Techniques based on microscopy and serology do not have sufficient sensitivity to detect Lxx when present in low titer in plant tissues (López et al., 2009; Abdalla, 2020). In these cases, therefore, the use of PCR is necessary. The conservation of the target gene sequence is an important identification criterion.

Genes used in detecting Lxx by PCR include genes involved in pathogenicity, anonymous regions of the genome, and ribosomal genes (Davis, 2000). The most used are primers designed from the intergenic transcribed spacer region (ITS) between the 16S and 23S rRNA genes of the bacterial rRNA operon (Astua-Monge, 1995; Davis et al., 2000; Fegan et al., 1998; Pan et al., 1998; Pan et al., 1999; Taylor, 2003; Faria et al., 2020). The presence of Lxx was not identified in the samples

(insect head, abdomen, and complete body), generating negative results.

Sugarcane thermotherapy is currently the primary management method for Lxx in Brazil. When immersed, the enzymes and proteins of the bacterial cell are denatured without harming the buds due to possible detection leaks and the consequent survival of the bacteria to thermotherapy, which can result in asymptomatic seedlings. Thus, none of the treatments adopted in thermotherapy was able to eliminate Lxx from the stalks, and the use of kasugamycin did not eliminate the bacteria but reduced the bacterial population (Dias et al., 2019).

These results confirm that nested-PCR is a valuable tool to detect the presence of this phytobacterium in cutlets that will be used as seedlings. PCR with all pairs of primers did not result in amplification in any insect samples collected from sugarcane fields inoculated with the bacterium, which indicates that they are not natural hosts of Lxx.

A survey on the incidence of RSD in commercial crops in the Center-South of Brazil showed that 9.4% of the 307 plots with cane-plant examined and 32% of the 50 plants sampled used seedlings contaminated with Lxx in areas of reform, revealing how widespread the disease is and the difficulty of effectively controlling the problem (Urashima et al., 2020).

A technique that is effective in detecting diseases is the PCR test, as it is based solely on the detection of the pathogen's DNA sequence, where the visualization of a fragment of a specific size after the PCR test would indicate the presence of the pathogen in the sample. Thus, this conclusion is important for practical purposes as it minimizes the possibility that any insect species, which are closely linked to sugarcane plantations, could be a host of the disease. On the other hand, sugarcane is the only natural host of Lxx is intriguing (Monteiro-Vitorello et al., 2004; Wu et al., 2018).

## 4. Conclusion

The insects *Diatraea flavipennella*, *Diatraea saccharalis*, *Mahanarva fimbriolata*, *Mahanarva posticata*, *Heterotermes tenuis*, in sugarcane fields do not present DNA of *Leifsonia xyli* subsp. *xyli* for the conditions tested.

Considering the conditions, we can suggest new experiments probably inoculating the *Leifsonia* directly in those insects and monitoring the bacteria persistence in insects.

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## References

Abdalla, M. M. E. (2020). Detection of the Bacteria *Leifsonia xyli* subsp. *xyli* (Davis) Using Lateral Flow Immunoassay Test and Modified Culture Media for Improving the diagnosis of Sugarcane Ratoon Stunt Disease (Doctoral dissertation, University of Gezira).

Carneiro, J.B., Barroso, L.M., Olivares, F.L. et al. (2021) Plant growth promotion of micropropagated sugarcane seedlings var. Co 412 inoculated with endophytic diazotrophic bacteria and effects on the Ratoon Stunting Disease. Australasian Plant Pathol. 50, 513–522.

Carvalho, G., Da Silva, T. G. E. R., Munhoz, A. T., Monteiro-Vitorello, C. B., Azevedo, R. A., Melotto, M., & Camargo, L. E. A. (2016). Development of a qPCR for *Leifsonia xyli* subsp. *xyli* and quantification of the effects of heat treatment of sugarcane cuttings on Lxx. Crop Protection, 80, 51-55.

Clavijo-Coppens, F., Ginet, N., Cesbron, S., Briand, M., Jacques, M. A., & Ansaldi, M. (2021). Novel Virulent Bacteriophages Infecting Mediterranean Isolates of the Plant Pest *Xylella fastidiosa and Xanthomonas albilineans*. Viruses, 13(5), 725.

Cursi, D. E., Castillo, R. O., Tarumoto, Y., Umeda, M., Tippayawat, A., Ponragdee, W., & Carneiro, M. S. (2022). Origin, Genetic Diversity, Conservation, and Traditional and Molecular Breeding Approaches in Sugarcane. In Cash Crops (pp. 83-116). Springer, Cham.

Davis, M. J., Gillaspie Jr, A. G., Vidaver, A. K., & Harris, R. W. (1984). *Clavibacter*: a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. International journal of systematic and evolutionary microbiology, 34(2), 107-117.

Davis, M. J., Rott, P., & Astua Monge, G. (1998). Nested, multiplex PCR for detection of both *Clavibacter xyli* subsp. xyli and *Xanthomonas albilineans* in sugarcane.

de Castro Moretti, F. R. (2018). Identification of candidate resistance metabolites to *Leifsonia xyli* subsp. *xyli* in sugarcane through metabolomic profiling. The Ohio State University.

de Souza, I. R. P., Barros, B. D. A., Lana, U. D. P., Rafael, H., Mendes, S., & Leite, N. (2013). Preservação de amostras e extração de DNA de Spodoptera frugiperda. Embrapa Milho e Sorgo-Boletim de Pesquisa e Desenvolvimento (INFOTECA-E).

Dias, V. D., Carrer, R., & Cunha, M. G. D. (2019). Comparison of *Leifsonia xyli* subsp. *xyli* molecular detection in heat-treated sugarcane setts. Pesquisa Agropecuária Tropical, 49.

Evtushenko, L. I., Dorofeeva, L. V., Subbotin, S. A., Cole, J. R., & Tiedie, J. M. (1962). *Leifsonia poae* gen. nov., sp. nov., isolated from nematode galls on *Poa annua* and reclassification of" *Corynebacterium aquaticum*.

Farahani, A. S., Taghavi, S. M., & Taher-Khani, K. (2015). Comparison of conventional, nested and real-time PCR for detection of the causal agent of ratoon stunt in Iran. Journal of Plant Pathology, 97(2).

Faria, R. S., Cia, M. C., Monteiro-Vitorello, C. B., Azevedo, R. A., & Camargo, L. E. A. (2020). Characterization of genes responsive to osmotic and oxidative stresses of the sugarcane bacterial pathogen *Leifsonia xyli* subsp. *xyli*. Brazilian Journal of Microbiology, 51(1), 77-86.

Fegan, M., Croft, B. J., Teakle, D. S., Hayward, A. C., & Smith, G. R. (1998). Sensitive and specific detection of *Clavibacter xyli* subsp. *xyli*, causal agent of ratoon stunting disease of sugarcane, with a polymerase chain reaction-based assay. Plant Pathology, 47(4), 495-504.

Garcia, J. F. (2013). Manual de Identificação das Pragas da Cana. Campinas, 219p.

Gillaspie, A. G. (1978). Ratoon stunting disease of sugarcane serology. Phytopathology, 68(3), 529-532.

López, M. M., Llop, P., Olmos, A., Marco-Noales, E., Cambra, M., & Bertolini, E. (2008). Are molecular tools solving the challenges posed by detection of plant pathogenic bacteria and viruses?. Current issues in molecular biology, 11(1), 13-46.

Madhaiyan, M., Poonguzhali, S., Lee, J. S., Senthilkumar, M., Lee, K. C., & Sundaram, S. (2010). Leifsonia soli sp. nov., a yellow-pigmented actinobacterium isolated from teak rhizosphere soil. International journal of systematic and evolutionary microbiology, 60(6), 1322-1327.

Marchetti, A. S.; Urashima, L. B. L. (2013) Incidência e gravidade de *Leifsonia xyli* subsp. *xyli* Infecção de cana-de-açúcar no Estado de São Paulo, Brasil. Jornal Phytopathol, v. 161, p. 478 – 484.

Mills, L., Leaman, T. M., Taghavi, S. M., Shackel, L., Dominiak, B. C., Taylor, P. W. J., & Teakle, D. S. (2001). *Leifsonia xyli*-like bacteria are endophytes of grasses in eastern Australia. Australasian Plant Pathology, 30(2), 145-151.

Monteiro-Vitorello, C. B., Camargo, L. E., Van Sluys, M. A., Kitajima, J. P., Truffi, D., Do Amaral, A. M., & Setúbal, J. C. (2004). The genome sequence of the gram-positive sugarcane pathogen *Leifsonia xyli* subsp. *xyli*. Molecular plant-microbe interactions, 17(8), 827-836.

Nordstedt, N. P., Roman-Reyna, V., Jacobs, J. M., & Jones, M. L. (2021). Comparative Genomic Understanding of Gram-Positive Plant Growth Promoting Leifsonia. Phytobiomes Journal, (ja).

Pan, Y. B., Grisham, M. P., & Burner, D. M. (1997). A polymerase chain reaction protocol for the detection of *Xanthomonas albilineans*, the causal agent of sugarcane leaf scald disease. Plant Disease, 81(2), 189-194.

Pan, Y. B., Grisham, M. P., Burner, D. M., Damann Jr, K. E., & Wei, Q. (1998). A polymerase chain reaction protocol for the detection of *Clavibacter xyli* subsp. *xyli*, the causal bacterium of sugarcane ratoon stunting disease. Plant disease, 82(3), 285-290.

Pan-Y. B.; Grisham, M. P.; Wei, Q. (2001) Diagnóstico por PCR de folha cana escaldadura e doenças soca nanismo. The Proceedings of the International Society of Sugar Cane Technologists, v. 24, p. 607 – 608.

Pelosi, C. S., Lourenço, M. V., Silva, M., Santos, A. Z., França, S. C., & Marins, M. (2013). Development of a Taqman real-time PCR assay for detection of *Leifsonia xyli* subsp *xyli*. Tropical Plant Pathology, 38, 343-345.

Ponte, E. C. D., Silveira, S. F. D., Carneiro Jr, J. D. B., & Lima, R. M. P. D. (2010). Incidência de *Leifsonia xyli* subsp. *xyli* em áreas de multiplicação de cana-de-açúcar no Espírito Santo, sul da Bahia e oeste de Minas Gerais. Summa Phytopathologica, 36, 313-321.

Reddy, G. S. N., Prabagaran, S. R., & Shivaji, S. (2008). *Leifsonia pindariensis* sp. nov., isolated from the Pindari glacier of the Indian Himalayas, and emended description of the genus *Leifsonia*. International journal of systematic and evolutionary microbiology, 58(9), 2229-2234.

Reddy, G. S. N., Prakash, J. S. S., Srinivas, R., Matsumoto, G. I., & Shivaji, S. (2003). *Leifsonia rubra* sp. nov. and *Leifsonia aurea* sp. nov., psychrophiles from a pond in Antarctica. International Journal of Systematic and Evolutionary Microbiology, 53(4), 977-984.

Rott, P. (Ed.). (2000). A guide to sugarcane diseases. Editions Quae.

Rott, P. C., Sood, S., Comstock, J. C., Gilbert, R. A., & Sandhu, H. S. (2018). [SC002-minor] Sugarcane Ratoon Stunting. EDIS, 2018.

Singh, P., Tsuji, M., Singh, S. M., & Takeuchi, N. (2020). Contrasting Patterns of Microbial Communities in Glacier Cryoconite of Nepali Himalaya and Greenland, Arctic. Sustainability, 12(16), 6477.

Steindl, D. R. L. (1976). The use of phase-contrast microscopy in the identification of ratoon stunting disease [bacterial disease, sugar cane; Queensland]. [Conference paper]. In 43. Conference. Queensland Society of Sugar Cane Technologists. Cairns, Queensland (Australia). 26 Apr 1976.

Taylor, P. W. J., Petrasovits, L. A., Der Velde, R. V., Birch, R. G., Croft, B. J., Fegan, M., & Brumbley, S. M. (2003). Development of PCR-based markers for detection of *Leifsonia xyli* subsp. *xyli* in fibrovascular fluid of infected sugarcane plants. Australasian Plant Pathology, 32(3), 367-375.

Taylor, P. W. J., Petrasovits, L. A., Der Velde, R. V., Birch, R. G., Croft, B. J., Fegan, M., & Brumbley, S. M. (2003). Development of PCR-based markers for detection of *Leifsonia xyli* subsp. *xyli* in fibrovascular fluid of infected sugarcane plants. Australasian Plant Pathology, 32(3), 367-375.

Tokeshi, H., & Rago, A. (1980). Doenças da cana-de-açúcar. Ed. Agronômica CERES.

Urashima, A. S., & Marchetti, L. B. (2013). Incidence and Severity of *Leifsonia xyli* subsp. *xyli* Infection of sugarcane in Sao Paulo State, Brazil. Journal of Phytopathology, 161(7-8), 478-484.

Urashima, A. S., Ganem Júnior, E. D. J., Marchetti, L. B. L., & Gagliardi, P. R. (2010). Incidence of Leifsonia xyli subsp xyli in sugarcane crops fron São Paulo state. Summa Phytopathologica, 36(4), 322-328.

Urashima, A. S., Silva, M. F., Coraini, N. F., & Gazaffi, R. (2020). Temporal incidence of *Leifsonia xyli* subsp. *xyli* in sugarcane propagating materials of Brazilian cultivars. Crop Protection, 128, 104976.

Wu, Q., Pan, Y. B., Zhou, D., Grisham, M. P., Gao, S., Su, Y., & Que, Y. (2018). A comparative study of three detection techniques for *Leifsonia xyli* subsp. *xyli*, the causal pathogen of sugarcane Ratoon stunting disease. BioMed research international, 2018.

Zavaglia, A. C., Cia, M. C., Popin, R. V., & Camargo, L. E. A. (2016). No alternative hosts of the sugarcane pathogen *Leifsonia xyli* subsp. *xyli* were identified among grass and non-grass species using novel PCR primers. Tropical Plant Pathology, 41(5), 336-339.

Zhang, X., Liang, Y., Song, X., Wang, Z., Zhang, B., Lei, J., & Yan, M. (2021). Changes in gene expression levels and chloroplast anatomy induced by *Leifsonia xyli* subsp. *xyli* in sugarcane. Journal of Plant Interactions, 16(1), 564-574.

Zhu, K., Yang, L. T., Li, C. X., Lakshmanan, P., Xing, Y. X., & Li, Y. R. (2021). A transcriptomic analysis of sugarcane response to *Leifsonia xyli* subsp. *xyli* infection. Plos one, 16(2), e0245613.