SHORT COMMUNICATION

e-Xtra*

Comparative Genomic Understanding of Gram-Positive Plant Growth-Promoting *Leifsonia*

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Accepted for publication 2 April 2021.

ABSTRACT

Root-associated bacteria play critical roles in shaping plant health for abiotic and biotic stress tolerance. The *Leifsonia* genus includes pathogenic and beneficial bacteria but limited research is available comparatively across species regarding their association with plants. We recently identified the plant growth-promoting *Leifsonia* sp. C5G2 isolated from the rhizosphere of *Plectranthus scutellarioides* (coleus) that increased stress tolerance and quality of water-stressed greenhouse ornamentals. Using this beneficial strain as a model, we performed comparative genomics with beneficial, commensal, and pathogenic *Leifsonia* spp. to better understand plant association and growth promotion under abiotic stress. Comparative phylogenetic analyses showed that beneficial and commensal *Leifsonia* spp. clustered separately from pathogenic *Leifsonia xyli* subsp. *xyli*. In addition, the beneficial or commensal strains share biosynthetic gene and orthologous protein clusters that are not shared with known phytopathogens in our analysis. The phytopathogenic *Leifsonia*

strains also share noticeably higher percent homology with plant-associated virulence factors in closely related *Clavibacter* spp. than the beneficial or commensal strains. Genome analysis supplemented with in vitro assays validated the findings that our model growth-promoting *Leifsonia* sp. strain C5G2 was highly prolific on diverse carbon sources. This is unlike known fastidious growth behaviors of phytopathogenic *Leifsonia* spp. Beneficial *Leifsonia* sp. C5G2 imparts water stress tolerance in plants, and multiple genes were identified in the genomes of beneficial *Leifsonia* strains that are potentially involved in bacterial osmotic stress and the production of osmoprotectants and vitamin B9. This work sheds light on the diversity of the genus *Leifsonia*, with a better understanding of potential mechanisms employed by poorly understood beneficial bacteria.

Keywords: agriculture, bacteriology, genomics, plants, rhizosphere and phyllosphere

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N. P. Nordstedt and V. Roman-Reyna contributed equally to this work.

Funding: Salaries and research support were provided, in part, by State and Federal funds appropriated to the Ohio Agricultural Research and Development Center (OARDC), The Ohio State University (OSU). Additional support was provided by the American Floral Endowment, OSU D. C. Kiplinger Floriculture Endowment, The Department of Horticulture and Crop Science, OSU Distinguished Fellowship, the OARDC Director's Graduate Associateship, the Altman Family Scholarship, the Department of Plant Pathology, C. Wayne Ellet Plant and Pest Diagnostic Clinic, and College of Food, Agricultural and Environmental Science at OSU; and funding from Ohio Department of Agriculture Specialty Crop Block Grant (AGR-SCG-19-03) and a United States Department of Agriculture–National Institute of Food and Agriculture (NIFA) award number 2018-67013-28490 through the National Science Foundation NIFA Plant Biotic Interactions Program to J. M. Jacobs.

*The e-Xtra logo stands for “electronic extra” and indicates that supplementary tables are published online.

The author(s) declare no conflict of interest.

Plant-associated microorganisms shape plant development and provide robust support for stress tolerance (Ngumbi and Kloepper 2016). Plant-growth-promoting rhizobacteria (PGPR), in particular, play critical roles in soil health, shaping the rhizospheric ecosystem, and supporting plant development (Lugtenberg and Kamilova 2009). Many PGPR allow plants to defend against pests and pathogens or tolerate environmental stresses such as heat, salinity, and drought (Goswami et al. 2016). Examples of beneficial PGPR are found across diverse bacterial genera (Goswami et al. 2016; Lugtenberg and Kamilova 2009) but little is known about plant-associated Gram-positive *Leifsonia* spp.

The genus *Leifsonia* comprises a group of Gram-positive bacteria belonging to the phylum *Actinobacteria*. *Leifsonia* spp. are poorly described among the actinomycetes, thus contributing to a limited number of reference genomes and little genetic information on these bacteria (Passari et al. 2015). Diverse *Leifsonia* spp. have beneficial and pathogenic relationships with plants (Kang et al. 2017, 2014; Monteiro-Vitorello et al. 2004, 2013; Wang et al. 2016). Most, although still limited, research has been in defining the biology and management of *Leifsonia xyli* subsp. *xyli*, which is a

xylem-dwelling fastidious plant pathogen of sugar cane (Monteiro-Vitorello et al. 2004, 2013; Wang et al. 2016). In our previous work, we identified *Leifsonia* sp. C5G2 as a PGPR. C5G2 was originally isolated from the rhizosphere of a severely water stressed *Plectranthus scutellarioides* (coleus) plant, and was shown to increase the stress tolerance and quality of greenhouse ornamental crops subjected to water stress (Nordstedt and Jones 2020). Cumulatively, this research provided insight into the diversity and biology of *Leifsonia*–plant associations. However, the genomics of this PGPR strain and other *Leifsonia*–plant associations remain to be explored.

In this work, we examined genomes belonging to beneficial, commensal, and plant-pathogenic *Leifsonia* strains. This work compares the genetic characteristics between these groups of bacteria, with an emphasis on understanding taxonomic diversity within the genus and factors that influence how different plant-associated bacteria interact with their host. In addition, we identified different elements likely contributing to the ability of beneficial *Leifsonia* spp. to stimulate plant growth, using the PGPR *Leifsonia* sp. C5G2 as a model. Here, we present the genetic characteristics of this important PGPR and identify potential mechanisms involved in bacterial osmotic stress tolerance and increasing plant water stress tolerance. The comparative genomic analyses also provide an important distinction of beneficial or commensal from phytopathogenic subgroups in the same genus and additionally show that there are few shared protein orthologs for plant association and pathogenesis between the genomes of the closely related *Clavibacter* and *Leifsonia* strains. Additionally, we show that phytopathogenic strains of *Leifsonia* share noticeably greater percent homology with functionally characterized virulence factors identified in *Clavibacter* strains than the beneficial or commensal *Leifsonia* strains. Our in vitro characterization describes antibiotic resistance, motility, and carbon source utilization capabilities in our model PGPR strain C5G2. Overall, this work provides valuable information on the metabolic and physiological capabilities of the genus *Leifsonia* and, in particular, the PGPR *Leifsonia* sp. C5G2.

MATERIALS AND METHODS

Sequencing and annotation of *Leifsonia* sp. C5G2. To understand the basis of plant association in *Leifsonia* spp., we sequenced the genome of the beneficial PGPR *Leifsonia* sp. C5G2 (Nordstedt and Jones 2020) for comparative analysis. The genomic DNA of C5G2 was extracted using the Quick-DNA Bacterial Miniprep kit (Zymo Research, Irvine, CA, U.S.A.) and submitted to CoreBiome (St. Paul, MN, U.S.A.) for sequencing. Libraries were prepared with the Nextera Library Prep kit (Illumina, San Diego, CA, U.S.A.) and sequenced on an Illumina NovaSeq using single-ended 1 × 100 reads with a NovaSeq SP flow cell.

Sequences were filtered for low quality (Q-score < 20) and length (<50), and adapter sequences were trimmed using cutadapt (v1.15). Sequences were then de novo assembled using SPAdes (v3.11.0) and contigs greater than 1,000 bases were retained as part of CoreBiome's proprietary pipeline. Contigs greater than 1,000 bases in length were then used in a QUAST (QUAST v4.5) analysis, and Prokka (v 1.12) was used to annotate genes within the assembly. PlasmidSPAdes was used for the detection of plasmids within the genome sequence of C5G2 (Antipov et al. 2016). BlastKOALA (v2.2) was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation and mapping (Kanehisa et al. 2016). The assembly is deposited in GenBank as Biosample SAMN14854757.

Taxonomic classification. Taxonomy of C5G2 was assigned using the Microbial Genomes Atlas (MiGA) server (Rodriguez-R et al. 2018). The phylogeny of *Leifsonia* spp. was validated by

calculating the Average Nucleotide Identity (ANI) based on BLAST and in silico DNA-DNA hybridization (isDDH) percentage of C5G2 and 30 additional actinomycetes. The ANI values were calculated using the Enveomics webtool (Rodriguez-R and Konstantinidis 2016) and isDDH percentages were calculated using the Genome-to-Genome Distance Calculator webtool (Meier-Kolthoff et al. 2013). The Newick tree files from the ANI results were used to generate the phylogenetic tree and edited in CLC genomics Workbench (v12).

Comparative genomic analyses. Bacterial genomes were selected as the core strains to represent beneficial (*Leifsonia* sp. C5G2 and *L. xyli* SE134), commensal (*Leifsonia* sp. M321_24_2 [M321]), and pathogenic (*L. xyli* subsp. *xyli* CTCB07 and *L. xyli* subsp. *xyli* gdw1) bacteria within the genus for comparative analyses in this study, unless otherwise noted. Carbohydrate-active enzymes (CAZymes) were predicted in the core genomes using the server dbCAN2 and the combined tools HMMER (E-value < 1e-15, coverage > 0.35), DIAMOND (E-value < 1e-102), and Hotpep (frequency > 2.6, hits > 6) (Zhang et al. 2018).

Genome structure and rearrangements were evaluated using Mauve (v2.4.0, 2015-02-25; ProgressiveMauve default alignments option) in the genomes for *Leifsonia* sp. C5G2, *Leifsonia* sp. M321, *L. xyli* SE134, *L. soli* DSM 23871, *Leifsonia* sp. 197AMF, and *L. xyli* subsp. *xyli* CTCB07 (Darling et al. 2004). The orthologous gene cluster (OGC) and gene ontology (GO) enrichment analysis was conducted on 20 actinomycetes using OrthoVenn2 with an E-value of 1e-2 and an inflation value of 1.5 (Xu et al. 2019). The results were plotted using UpSetR (Conway et al. 2017). The additional sequences were included in this analysis to characterize the differences in GO between the different phylogenetic groups identified in genus *Leifsonia* and the closely related genus *Clavibacter*. Biosynthetic gene clusters (BGCs) involved in the synthesis of secondary metabolites were predicted using antiSMASH (v5.0) (Blin et al. 2019) across 11 reference sequences.

Annotation files for the known PGPR strains C5G2 and SE134 were searched manually to identify genes potentially involved in plant growth promotion, particularly in regard to osmotic stress.

Leifsonia genomes were also investigated for their homology to virulence factors involved in plant pathogenicity. Functionally characterized virulence factors from the closely related plant-pathogenic strain *Clavibacter michiganensis* subsp. *michiganensis* NCPPB382 were used as reference sequences to determine homology (Eichenlaub and Gartemann 2011; Thapa et al. 2017). Whole-genome sequences for beneficial (*Leifsonia* sp. C5G2 and *L. xyli* SE134), commensal (*Leifsonia* sp. M321), and pathogenic (*L. xyli* subsp. *xyli* CTCB07, *L. xyli* subsp. *xyli* gdw1, and *L. xyli* subsp. *cynodontis* DSM 46306) strains were used to search for homology against the reference protein sequences for the NCPPB382 genes *celA*, *pat-1*, *chpC*, *chpG*, *ppaA*, and *ppaC*. Whole-genome sequences for the nonpathogenic endophyte *C. michiganensis* CASJ009 and the pathogenic *C. michiganensis* subsp. *michiganensis* UF1 were also used for comparison. The webserver tblastn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al. 1997) was used to determine percent amino acid similarity of each virulence factor to protein sequences in each whole-genome sequence.

In vitro characterization of *Leifsonia* sp. C5G2. We evaluated the antibiotic resistance of our model strain C5G2 in vitro according to Krumperman (1983). Bacterial growth was evaluated with 1×- and 0.5×-strength antibiotic, with 1× concentrations as follows: rifampicin (50 µg/ml), streptomycin (50 µg/ml), kanamycin (50 µg/ml), ampicillin (100 µg/ml), chloramphenicol (25 µg/ml), gentamicin (25 µg/ml), carbenicillin (100 µg/ml), and tetracycline (5 µg/ml). Half-strength Luria-Bertani agar medium was prepared and supplemented with each of the individual antibiotics. Bacterial

inoculum was prepared by collecting bacterial cells via centrifugation and resuspending them in phosphate-buffered saline (PBS) buffer. A 5-μl aliquot of inoculum was struck out onto each of the antibiotic plates in triplicate ($n = 3$) and incubated at 28°C for 96 h. Sterile PBS without bacteria was used as the control. Bacterial growth on each plate was then recorded as yes or no.

Sole carbon source utilization was evaluated according to Pridham and Gottlieb (1948). Minimal media agar plates were prepared, and each carbon source was added individually after autoclaving for a final concentration of 1% wt/vol (wt/wt for glycerol). C5G2 was tested for growth on fructose, galactose, glucose, glycerol, cellobiose, sucrose, mannitol, and ribose, each as the sole source of carbon because of their role in the metabolically important pentose phosphate pathway and glycolysis. Inoculum was prepared and struck onto plates as described previously for the antibiotic resistance experiment. Plates were incubated at 28°C for 96 h, and bacterial growth on each plate was then recorded as yes or no.

Swimming motility of C5G2 was tested in semisolid NYGA media according to Jacobs et al. (2015). Overnight culture of C5G2 was diluted to optical density at 595 nm = 0.8 and the inoculum was stabbed in NYGA media in triplicate ($n = 3$). The cultures were incubated at 28°C for 96 h and turbidity surrounding the stab was evaluated. Visible turbidity that is away from the initial site of the stab inoculation is an indication of the swimming motility capabilities of the bacterium.

To determine the doubling time and growth rate of C5G2, the bacterium was grown in two common laboratory media, nutrient broth and peptone sucrose broth. Inoculum was prepared by resuspending colonies in sterile water and then adjusting the absorbance to optical density at 600 nm = 0.01. In a 96-well microtiter plate, 10 μl of C5G2 inoculum was added to 90 μl of each separate media, with five replicates per media type ($n = 5$). Using a spectrophotometric plate reader (Synergy H1; Biotek, Winooski, VT, U.S.A.), absorbance was measured every 30 min for 40 h with constant shaking (200 rpm) at 28°C.

RESULTS

Genome assembly and annotation of *Leifsonia* sp. C5G2.

De novo assembly of the sequencing reads of C5G2 resulted in 35 contigs with >1,000-bp length. Accordingly, the draft genome of C5G2 consists of 4,091,029 bp, with an average GC content of 70.4%. No plasmids were identified within the genome. According to the Prokka annotation, the C5G2 genome has 4,018 predicted genes, of which 3,915 (97.44%) have a protein sequence (Table 1). KEGG analysis identified 1,872 genes, 47% of all coding sequences, involved in metabolic pathways. The largest numbers were involved in carbohydrate metabolism (13.7%), signaling and cellular processes (11.9%), protein family genetic information processing (11.2%), or unclassified functions (including ‘Unclassified: metabolism’, ‘Unclassified: signaling and cellular processes’, ‘Unclassified: genetic information processing’, and ‘Unclassified’ functional categories) (14.2%) (Supplementary Table S1).

Taxonomic classification of *Leifsonia* sp. C5G2. The determine the taxonomic classification of C5G2, we used the MiGA webserver because of its ability to compare the ANI of a query sequence across the NCBI prokaryotic database for identification. Results from MiGA showed that C5G2 belongs to the genus *Leifsonia* ($P = 0.038$), and to a species not represented in the database ($P = 0.0029$). Therefore, we used isDDH and ANI for further taxonomic evaluation because both are standard methods for species identification of bacteria, and a minimum of 94% ANI and 70% isDDH are the determined thresholds to classify two strains as the same species (Chun et al. 2018). Our comprehensive ANI and

isDDH results corroborated these findings, because C5G2 did not meet these thresholds with any other strain with a species identification (Supplementary Table S2). Our phylogenetic analysis showed that C5G2 clustered with the commensal bacteria *Leifsonia* sp. M321 24_2 (M321) and the PGPR *L. xyli* SE134 (Fig. 1), and further analysis showed that C5G2 shares 85 to 86% ANI and 28 to 29% isDDH similarity with M321 and SE134 (Supplementary Table S2). Beyond this cluster, the closest phylogenetic relatives are *L. soli* and multiple *Leifsonia* sp. strains. Notably, C5G2 is phylogenetically distant from the known *Leifsonia* phytopathogens *L. xyli* subsp. *xyli* gdw1, *L. xyli* subsp. *xyli* CTCB07, and *L. xyli* subsp. *cynodontis* DSM 46306 (Fig. 1; Supplementary Table S2).

Comparative genome analyses. In addition to providing evidence that *Leifsonia* sp. C5G2 likely belongs to a novel species, our ANI and isDDH data shed light on the diversity between *Leifsonia* spp. Both data sets complement each other, providing similar results in regard to *Leifsonia* phylogeny (Supplementary Table S2). The *Leifsonia* sp. strains 157MF, 197AMF, 466MF, 467MF, 509MF, and 98AMF are nearly identical because their ANI and isDDH values are 100%. However, the other *Leifsonia* sp. strains C5G2, M321, and PS1209 do not share >94% ANI or >70% isDDH similarity with any *Leifsonia* strain in this analysis. *Leifsonia* sp. 21MFCrub1.1 meets the criteria to be the same species as the bacteria identified previously as *L. soli* DSM 23871 because the strains share 98.2% ANI and 84.7% isDDH similarity. Additionally, *L. naganoensis* DSM 15166 and *L. aquatica* ATCC 14665 share 97.3% ANI and 76.7% isDDH similarity. The ANI-isDDH dual matrix also confirms that the three *L. xyli* subsp. *xyli* strains are the same species because they share 99.9 to 100% ANI and isDDH similarity. However, *L. xyli* subsp. *cynodontis* DSM 46306 and *L. xyli* SE134 do not share a species relationship with *L. xyli* subsp. *xyli* or each other, as determined by their shared ANI and isDDH values, which ranged from 81.7 to 92.9% and 23.0 to 52.6%, respectively. Notably, several of the *C. michiganensis*

TABLE 1
Genome features of *Leifsonia* sp. C5G2

Features ^a	Statistics
Assembly	
Genome coverage	60×
Number of bases	4,091,029
Number of contigs	35
N ₅₀ read length	228,548
GC content (%)	70.40
Annotation	
Open reading frames	4,018
Number of CDSs	3,967
Protein-coding genes	3,915
tRNA genes	45
ncRNAs	3
Pseudogenes	52
Secondary metabolite BGCs	6
Genes assigned to KEGG	1,773
CAZymes	109
Secreted proteins	374

^a CDSs = coding sequences, BGCs = biosynthetic gene clusters, KEGG = Kyoto Encyclopedia of Genes and Genomes, and CAZymes = carbohydrate-active enzymes.

subspecies do not meet the criteria (>94% ANI or >70% isDDH similarity) to be considered the same species (Supplementary Table S2).

Progressive Mauve alignments were used to evaluate genome rearrangements between strains C5G2, M321, SE134, DSM 23871, 197AMF, and CTCB07 (Darling et al. 2004). *L. xyli* subsp. *xyli* gdw1 was not included in the analysis due to Mauve locally collinear block (LCB) calculating errors. The Mauve alignments showed a total of six LCBs (Fig. 2). The strains SE134 and M321 had six LCBs, while the other *Leifsonia* sp. strains had five LCBs. *L. xyli* subsp. *xyli* CTCB07 had three blocks, with white spaces indicating no detected synteny with the other strains. All LCBs had low similarities among the six *Leifsonia* strains, despite being identified as conserved regions by Mauve. Similar to *C. michiganensis*, *Leifsonia* genomes had few LCB rearrangements (Lu et al. 2018). Moreover, each strain had unique genomic regions representing at least 20% of their genome.

Carbohydrate metabolism plays a central role in bacterial biology, and different characteristics of this metabolism can highlight distinguishing characteristics between bacterial strains. Carbohydrate metabolism is significantly enriched in the PGPR strains C5G2 and SE134 and the commensal strain M321 ($P < 0.001$). However, this was not the case for the phytopathogenic strains

L. xyli subsp. *xyli* gdw1 and *L. xyli* subsp. *xyli* CTCB07 ($P = 1$). Overall, there are noticeable differences in which KEGG functional categories were most enriched between the beneficial or commensal and phytopathogenic strains (Supplementary Table S1). Additionally, our comparative analysis between the PGPR strains C5G2 and SE134, the commensal strain M321, and the phytopathogens CTCB07 and gdw1 identified 63 singlest or groups of CAZymes encoded in at least one of the genomes (Supplementary Table S3). Most of the annotations belong to nonsecreted CAZymes, as opposed to secreted ones. A majority of the CAZymes were identified only in the genomes of C5G2, SE134, and M321. These included carbohydrate esterases, glycoside hydrolases (GHs), and glycosyl transferases (GTs). CAZymes identified exclusively in the PGPR strains C5G2 and SE134 include a variety of GHs. Nonsecreted carbohydrate-binding modules (CBM) and secreted GHs were exclusive to the genome of C5G2. A nonsecreted GH and GT and a secreted CBM and GH were identified exclusively in the two phytopathogenic strains. The CAZymes belonging to CBM, GHs, and GTs were identified in all five genomes (Supplementary Table S3).

The species evaluated in the OGC GO enrichment analysis using OrthoVenn2 formed a total of 5,047 clusters, with 5,000 orthologous clusters shared between at least two strains. Five different

Fig. 1. Phylogram based on average nucleotide identity (ANI) to describe the relationship of beneficial, commensal, and pathogenic *Leifsonia* spp. The ANI based on BLAST was calculated using whole genome sequences and distance was estimated using neighbor-joining as the clustering method. Our model beneficial strain C5G2 is in bold letters, and bacterial strains are identified as beneficial (purple square), commensal (orange square), or pathogenic (red square). The NCBI GenBank accession number for each genome with the ANI statistics are indicated in Supplementary Table S1.

phylogenetic clusters (PCs) were determined based on their position in the phylogenetic tree and in connection with C5G2, as indicated with the blue boxes in Figure 3. The PCs consisted of all 20 actinomycetes (PC 1), *Leifsonia* strains without *Clavibacter* sp. (PC 2), *Leifsonia* spp. without *L. xyli* subsp. *xyli* strains (PC 3), only commensal and PGPR strains (PC 4), and the two known PGPR strains and the closely related commensal strain M321 (PC 5). The most abundant orthologs were among PC 1 containing all 20 strains, which likely includes orthologs from the core bacterial genome, followed by the *Clavibacter*-specific cluster, and the third most abundant being among the commensal or beneficial *Leifsonia* strains (PC 3) (Fig. 3). Overall, 26.2% of the total OGCs were shared among all of the species evaluated (PC 1), whereas 4.3% were unique to the group of only *Leifsonia* spp. (PC 3) and 1.1% of the total OGCs were unique to the cluster of beneficial or commensal strains (PC 5) (Fig. 3). The GO annotations were highly diverse between each of the PCs, with few genes unique or enriched to each PC (Supplementary Table S4). For example, the GO response to antibiotic were enriched in PC 1 and PC 4, whereas response to copper ion was unique to PC 5 (Fig. 4).

Although multiple PGPR, including our model strain C5G2, have been reported to increase plant growth under water stress, these bacteria must first be able to withstand the onset of osmotic stress in order to increase stress tolerance of their plant host (Ngumbi and Kloepper 2016). Microbes have developed mechanisms such as maintaining an optimal cytoplasmic ion concentration (Booth 2014) to counteract the onset of water stress. Genes potentially involved in bacterial osmotic stress tolerance and increasing plant water stress tolerance through the synthesis of tryptophan, folic acid, and the osmoprotectants trehalose and proline were identified within the genomes of the PGPR strains C5G2 and SE134 (Table 2). Additionally, beneficial *Leifsonia* spp. that promote plant growth displayed gene clusters associated with water stress tolerance (Fig. 4). Furthermore, genes encoding for multiple potassium importers (*ktrAB* and *kdpABCDE*), sodium antiporters (*nhaK*), and the *MtrAB* two-component system were identified in the genomes of C5G2 and SE134 (Gouda et al. 2001; Hahne et al. 2010; Kang et al.

2016; Kixmüller and Greie 2012; Möker et al. 2004; Szollosi et al. 2016) (Table 2). Rhizospheric bacteria must also cope with highly oxidative conditions in the rhizosphere because plants produce different reactive oxygen species (ROS) as a defense mechanism during early stages of plant-microbe interactions (Bible et al. 2016; Colburn-Clifford et al. 2010). PC 5, which contains *Leifsonia* strains C5G2, M321, and SE134, included multiple cluster-specific orthologs involved in ROS defense and detoxification (Fig. 4). Additionally, multiple genes were identified in the genome annotations for C5G2 and SE134 (Table 2).

After colonization, PGPR can increase plant water stress tolerance through different mechanisms, including the production of osmoprotectants and vitamins and modulation of phytohormone levels (Ngumbi and Kloepper 2016). The genomes of C5G2 and SE134 encode for multiple genes involved in the synthesis and transport of the osmoprotectant trehalose (*treS*, *treYZ*, *otsAB*, and *proABC*) that can be utilized by plants during times of severe water stress to increase plant stress tolerance (De Smet et al. 2000; Eida et al. 2020; Julca et al. 2012; Paul et al. 2008; Roca et al. 2013; Vilchez et al. 2016) (Table 2). Genes encoding for the synthesis of folic acid (vitamin B11), which belongs to a class of essential cofactors involved in enzymatic reactions that form essential amino acids in plants (Burgess et al. 2009; Hanson and Gregory 2011), were also identified in the genomes of C5G2 and SE134 (Table 2). Additionally, the genomes of C5G2 and SE134 encode for tryptophan (Trp) biosynthesis, the precursor of the plant phytohormone indole-3-acetic acid (IAA) (Lugtenberg and Kamilova 2009; Niu et al. 2019); however, they do not encode for enzymes responsible for converting Trp to IAA (Fig. 4; Table 2).

We used antiSMASH to identify genomic regions encoding for potential secondary metabolite BGCs. The antiSMASH analysis identified six BGCs for C5G2 (Supplementary Table S5). From the six BGCs, lasalocid and heme D1 were unique to the C5G2 genome. The genome of C5G2 shares the BGC for alkylresorcinol with the PGPR SE134, the potato endophyte PS1209, and the commensal strains M321 and DSM 23871. Alkylresorcinols are phenolic lipids synthesized by bacteria during cyst formation, a defense

Fig. 2. Multiple genome alignment of C5G2 to the closest *Leifsonia* genomes shows low levels of similarities in genomic regions despite being identified as conserved regions. Each genome in the alignments is laid out horizontally. Blocks with same color and connected with lines are considered locally collinear blocks (LCBs) with LCBs as colored rectangles. Average sequence similarities within an LCB are proportional to the amount of color in the block. On the left, a phylogenetic tree indicates the relation among the compared strains and the type of interaction with plants. The *Leifsonia xyli* SE134 genome was used as the reference. Genome alignments and visualization were done using Mauve. Our model beneficial strain C5G2 is in bold letters, and bacterial strains are identified as beneficial (purple square), commensal (orange square), or pathogenic (red square).

response to survive under unfavorable environmental conditions (Romero et al. 2013). Notably, the genome of C5G2 does not share any BGCs with the two known *Leifsonia* phytopathogenic strains CTCB07 and gdw1.

In addition to evaluating genes potentially involved in plant growth promotion in the PGPR strains C5G2 and SE134, we investigated beneficial, commensal, and pathogenic *Leifsonia* genomes for homology based on amino acid similarity to plant-associated virulence factors. Overall, we evaluated homology of six virulence factors that have been functionally characterized in the closely related strain *C. michiganensis* subsp. *michiganensis* NCPPB 382. Of the six *Leifsonia* strains evaluated, the three beneficial or commensal strains do not share significant similarity with any of the six virulence factors, with the exception of SE134, which shares only 45% amino acid similarity to the *celA* gene. In contrast, each of the three pathogenic strains share >60% amino acid similarity to the *celA* gene and >50% amino acid similarity to the *pat1* and *chpG*

genes. Additionally, *L. xyli* subsp. *xyli* gdw1 shares >50% amino acid similarity to the *chpC* gene (Fig. 5). We included other commensal and pathogenic *Clavibacter* strains for comparison in our analysis. *C. michiganensis* CASJ009 is documented as a nonpathogenic endophyte of tomato (Thapa et al. 2017), and does not share significant similarity to the *celA* gene but does share >50% amino acid similarity to the other virulence factors in the analysis, except for *chpC*. The pathogenic strain *C. michiganensis* subsp. *sepedonicus* ATCC33113 shares >50% amino acid similarity to each of the six virulence factors, with >88% similarity to *celA*, *pat1*, and *chpC*. The pathogenic strain *C. michiganensis* subsp. *michiganensis* UF1 shares near-identical protein homology with all six virulence factor reference sequences of *C. michiganensis* subsp. *michiganensis* NCPPB 382 (Fig. 5).

In vitro characterization. To evaluate C5G2 antibiotic resistance, we grew the strain on media containing each of eight different antibiotics. C5G2 exhibited moderate antibiotic resistance and

Fig. 3. Comparative genomic analysis identifies subgroup-specific orthologs. Orthologous gene cluster (OGC) comparisons were made among 19 genomes closely related to *Leifsonia* sp. C5G2. Cluster count indicates the OGCs shared between the strains. The connected black dots indicate the strains that share the same cluster. On the left, a phylogenetic tree indicates the relation among the compared strains and the type of interaction with plants. On the right, the histogram indicates the number of proteins per genome. Blue bars indicate the selected clusters for gene ontology enrichment, and all contain C5G2 for comparison. Selected clusters are also indicated in the phylogenetic tree as blue squares. Phylogenetic cluster (PC) 1 shared 1,053 clusters, PC 2 shared 129, PC 3 shared 430, PC 4 shared 50, and PC 5 shared 34. Bacterial strains are identified as beneficial (purple square), commensal (orange square), or pathogenic (red square).

GO Annotation	PC #1	PC #2	PC #3	PC #4	PC #5
GO:0015144 Carbohydrate transmembrane transporter activity	1			2	
GO:0008324 Cation transmembrane transporter activity			2	2	
GO:0016491 Oxidoreductase activity	3		5	4	1
GO:0004601 Peroxidase activity			1	1	
GO:0051920 Peroxiredoxin activity			1	1	
GO:0004784 Superoxide dismutase activity	1				
GO:0004834 Tryptophan synthase activity	2				
GO:0006865 Amino acid transport	2		1	3	
GO:0005975 Carbohydrate metabolic process	5		2	5	
GO:0008643 Carbohydrate transport		1	2	4	
GO:0006878 Cellular copper ion homeostasis			1	1	
GO:0010106 Cellular response to iron ion starvation	2				
GO:0071732 Cellular response to nitric oxide				2	
GO:0030245 Cellulose catabolic process				2	
GO:0006935 Chemotaxis		1	2	1	
GO:0042732 D-xylose metabolic process			1	2	
GO:0015753 D-xylose transmembrane transport			1	1	
GO:0045226 Extracellular polysaccharide biosynthetic process	1				
GO:0006012 Galactose metabolic process				1	
GO:0006096 Glycolytic process	12		3	5	
GO:0009098 Leucine biosynthetic process	4				
GO:0006552 Leucine catabolic process			2	2	
GO:0097054 L-glutamate biosynthetic process	1				
GO:0019594 Mannitol metabolic process	1			1	
GO:0009405 Pathogenesis	5			1	
GO:0009297 Pilus assembly			1	1	
GO:0071805 Potassium ion transmembrane transport	1		1	1	
GO:0006813 Potassium ion transport	3				
GO:0009306 Protein secretion	1		4	4	1
GO:0015031 Protein transport	2		2	2	
GO:0006885 Regulation of pH			1	1	
GO:0019430 Removal of superoxide radicals	1				1
GO:0010044 Response to aluminum ion			1		
GO:0046677 Response to antibiotic	7	1	5	7	1
GO:0046685 Response to arsenic-containing substance	1		1	1	1
GO:0009409 Response to cold	1				
GO:0046688 Response to copper ion					2
GO:0042493 Response to drug	1				
GO:0009408 Response to heat	4				
GO:0009635 Response to herbicide	1				
GO:0033194 Response to hydroperoxide	1				
GO:0009624 Response to nematode			1		
GO:0010045 Response to nickel cation			1	1	1
GO:0006979 Response to oxidative stress	3	2			
GO:0009314 Response to radiation	2				
GO:0009636 Response to toxic substance			1	1	
GO:0009697 Salicylic acid biosynthetic process	1				
GO:0006814 Sodium ion transport				1	
GO:0009088 Threonine biosynthetic process	5				
GO:0000162 Tryptophan biosynthetic process	5				

Fig. 4. Orthologous gene clusters (OGCs) potentially involved in bacterial osmotic stress tolerance or plant growth promotion between phylogenetic clusters (PC). All PC contain C5G2 for comparisons. OGCs potentially involved in bacterial osmotic stress tolerance or plant growth promotion were searched across all PC. The number corresponding to each cell is the number of that gene ontology (GO) annotation within each PC, represented by a red-blue color scale. Gray cells indicate that GO annotation was not identified within that PC.

was able to grow on 1×-strength kanamycin and 0.5×-strength kanamycin, ampicillin, chloramphenicol, gentamycin, and carbenicillin. C5G2 was unable to grow on either concentration of rifampicin, streptomycin, or tetracycline. C5G2 tested negative for motility, as indicated by its lack of turbid growth outside of the inoculated stab zone (data not shown). This result is supported by the lack of genes encoding for flagellar assembly or motility identified in the genome of C5G2 (Supplementary Table S6).

Our in vitro carbon source utilization assay supported our KEGG analysis findings, because C5G2 had a robust carbon metabolism and was able to grow on media containing fructose, galactose, glucose, glycerol, cellobiose, sucrose, mannitol, and ribose, each as the sole source of carbon. The nonfastidious nature of C5G2 in solid media was also validated in liquid culture. C5G2 growth took less than 8 h to reach the midlog phase and the growth rate was 0.25 ± 0.064 in nutrient broth and 0.58 ± 0.049 in peptone sucrose broth with constant shaking (200 rpm) at 28°C.

DISCUSSION

The genus *Leifsonia* belongs to the actinomycetes and includes beneficial, commensal, and pathogenic strains. However, the Gram-positive actinomycetes are highly underexplored and the understanding of how Gram-positive bacteria suppress host defense and induce pathogenicity still remains largely unknown (Hogenhout and Loria 2008; Thapa et al. 2017). Mechanistic understanding of pathogenicity has been more extensively studied in the closely related genus *Clavibacter* than in *Leifsonia* (Chalupowicz et al. 2012; Savidor et al. 2012; Thapa et al. 2017; Xu et al. 2012; Załuga et al. 2014). Therefore, we used genomic and functional information known about the closely related pathogenic *Clavibacter* spp. to drive our analysis of comparing pathogenicity traits between beneficial, commensal, and pathogenic *Leifsonia*.

Strains of *Clavibacter* and *Leifsonia* are shown to be host-specific at the species or subspecies level (Hogenhout and Loria 2008).

TABLE 2
Genes identified in the Prokka genome annotation for *Leifsonia* sp. C5G2 and *Leifsonia xyli* SE134 potentially involved in bacterial osmotic stress tolerance and increasing plant water stress tolerance^a

Putative function, gene	C5G2	SE134	Reference
Bacterial osmotic stress			
<i>ktrAB</i>	X	X	Szollosi et al. 2016
<i>kdpABCDE</i>	X	X	Kixmüller and Greie 2012
<i>nhaG</i>	X	—	Gouda et al. 2001
<i>nhaK</i>	X	X	Hahne et al. 2010
<i>mtrAB</i>	X	X	Möker et al. 2004
Antioxidant production and reactive oxygen species detoxification			
<i>soda</i>	X	X	Inaoka et al. 1999
<i>katA</i>	X	X	Cosgrove et al. 2007
<i>bcp</i>	X	X	Jeong et al. 2000
<i>osmC</i>	X	X	Si et al. 2019
<i>cpo</i>	X	X	Wiesner et al. 1988
<i>bpoC</i>	X	—	Villalobos et al. 2019
<i>efeB</i>	X	X	Villalobos et al. 2019
<i>efeN</i>	X	X	Villalobos et al. 2019
<i>hmp</i>	X	—	Hu et al. 1999
<i>btuE</i>	X	X	Arenas et al. 2010
<i>gsiABCD</i>	X	—	Smirnova et al. 2015
<i>ohrB</i>	X	X	Zuber 2009
<i>ohrR</i>	X	X	Fontenelle et al. 2011
Root surface adhesion			
<i>xerC</i>	X	X	Martínez-Granero et al. 2005
<i>xerD</i>	X	X	Martínez-Granero et al. 2005
Osmoprotectant biosynthesis			
<i>treS</i>	X	X	Paul et al. 2008
<i>treYZ</i>	X	X	Paul et al. 2008
<i>otsAB</i>	X	X	Paul et al. 2008
<i>proABC</i>	X	X	Bhargava 2006
Tryptophan biosynthesis			
<i>trpABCDE</i>	X	X	Niu et al. 2019
Folic acid biosynthesis			
<i>folEBKPCA</i>	X	X	Burgess et al. 2009

^a Presence of each gene is indicated with an X.

Such is the case with *Clavibacter* subspecies specificity to tomato and potato and phytopathogenic *L. xyli* subsp. *xyli* strains CTCB07 and *gdw1* specificity to sugarcane (Monteiro-Vitorello et al. 2004; Wang et al. 2016). *L. xyli* subsp. *xyli* has a historical association and specificity with grasses (Brumbley et al. 2006) and the related strain *L. xyli* subsp. *cynodontis* DSM46306 is a vascular pathogen of Bermuda grass (Monteiro-Vitorello et al. 2013). C5G2 is unique in that it has been shown to associate with multiple dicotyledonous species (Nordstedt and Jones 2020), and the phylogenetically close PGPR strain SE134 has been shown to increase plant growth of cucumber, radish, rice, and tomato (Kang et al. 2014, 2017). This work describes the genetic characteristics of the novel PGPR *Leifsonia* sp. C5G2 and compares these characteristics to other beneficial, commensal, and pathogenic strains within the same genus.

Our phylogenetic analysis shows that C5G2 clusters closely with the PGPR SE134 and the commensal strain M321, and it is relatively distantly related to the phytopathogenic *L. xyli* subsp. *xyli* strains (Fig. 1). Our work used a dual ANI-isDDH matrix to confirm taxonomic relationships within the genus. This analysis shows that the genus *Leifsonia* is extremely diverse, and many strains with the 'species' designation (*Leifsonia* sp.) do belong to novel species within the genus due to their low shared ANI and isDDH values (Supplementary Table S2) (Colston et al. 2014; Konstantinidis and Tiedje 2005). Additionally, this analysis confirms the shared species relationship between publicly available *L. xyli* subsp. *xyli* strains but provides evidence that *L. xyli* SE134 and *L. xyli* subsp. *cynodontis* DSM 46306 are not the same species as *L. xyli* subsp. *xyli* strains. Our model PGPR strain C5G2 is similar in that it is phylogenetically close to *L. xyli* SE134 and *Leifsonia* sp. M321 relative to other *Leifsonia* strains (Fig. 1); however, it is still distinctly its own

species according to the dual ANI-isDDH data (Supplementary Table S2). The combination of ANI and isDDH data proved to be a useful tool to confirm the taxonomic relationships between species, because both data sets corroborated the other in regard to the *Leifsonia* strains complexity. Notably, the ANI and isDDH data also provide evidence that several of the *C. michiganensis* subspecies do not meet the criteria to be considered the same species, suggesting further work in understanding the taxonomy of these closely related strains.

The unique genomic regions identified in the Mauve alignments and the low similarity scores for all LCBs likely contribute to the low ANI values between the different *Leifsonia* sequences. Moreover, few LCBs across *Leifsonia* spp. indicates that the gene content is different across genomes. This is clear for the strain *L. xyli* subsp. *xyli* CTCB07 and *Leifsonia* sp. C5G2. These results reinforce the idea that the *Leifsonia* genomes have high levels of diversity and genome plasticity, providing justification for further exploration of these species (Fig. 2).

Plant-pathogenic Gram-negative bacteria often utilize the type III secretion system for virulence. However, due to their difference in cellular membranes, Gram-positive bacteria are not known to utilize most type-secretion systems, and the mechanistic understanding of Gram-positive virulence is still largely unknown. Fortunately, work in recent years has functionally characterized several genes involved in *Clavibacter* plant virulence (Eichenlaub and Gartemann 2011; Thapa et al. 2017). Due to the close relationship between the genera *Leifsonia* and *Clavibacter*, our genome analysis evaluated homology between *Clavibacter* virulence factor reference sequences and beneficial, commensal, and pathogenic *Leifsonia* genome sequences. Notably, the beneficial or commensal *Leifsonia* strains did not

Gene	<i>L. sp.</i> C5G2	<i>L. xyli</i> SE134	<i>L. sp.</i> M321	<i>Lxx</i> CTCB07	<i>Lxx</i> gdw1	<i>Lxc</i> DSM46306	<i>Cm</i> CASJ009	<i>Cms</i> ATCC33113	<i>Cmm</i> UF1
<i>celA</i>	NS	45%	NS	63%	63%	65%	NS	89%	99%
	NS	0.013	NS	4x10 ⁻¹³¹	4x10 ⁻¹³¹	7x10 ⁻¹³³	NS	0.0	0.0
	NS	12%	NS	61%	61%	61%	NS	99%	100%
<i>pat1</i>	NS	NS	NS	57%	57%	55%	52%	88%	100%
	NS	NS	NS	4x10 ⁻²⁷	1x10 ⁻²⁷	1x10 ⁻³²	8x10 ⁻⁴⁸	4x10 ⁻¹³⁰	1x10 ⁻¹⁷⁴
	NS	NS	NS	83%	77%	89%	89%	90%	100%
<i>chpC</i>	NS	NS	NS	43%	51%	49%	48%	93%	100%
				1x10 ⁻²²	2x10 ⁻²⁰	4x10 ⁻²⁹	2x10 ⁻³⁰	1x10 ⁻¹⁷²	0.0
				95%	74%	97%	93%	100%	100%
<i>chpG</i>	NS	NS	NS	52%	52%	54%	52%	51%	99%
				6x10 ⁻¹⁸	1x10 ⁻²⁰	1x10 ⁻⁴²	2x10 ⁻³⁴	2x10 ⁻³⁹	6x10 ⁻¹⁷⁵
				82%	73%	88%	97%	84%	100%
<i>ppaA</i>	NS	NS	NS	41%	41%	43%	63%	50%	98%
				2x10 ⁻¹⁷	2x10 ⁻¹⁷	7x10 ⁻¹⁹	1x10 ⁻⁷⁶	6x10 ⁻⁴³	0.0
				81%	81%	68%	84%	80%	100%
<i>ppaC</i>	NS	NS	NS	40%	40%	37%	60%	69%	100%
				4x10 ⁻¹⁰	4x10 ⁻¹⁰	1x10 ⁻⁹	4x10 ⁻⁵⁸	3x10 ⁻⁷¹	2x10 ⁻¹⁷⁶
				75%	64%	70%	75%	78%	100%

Fig. 5. Percent amino acid similarity, BLAST Expect (E) value, and query coverage of plant-associated virulence factors within *Leifsonia* and *Clavibacter* genomes. Genomes of beneficial (*Leifsonia* sp. C5G2 and *Leifsonia xyli* SE134), commensal (*Leifsonia* sp. M321 and *Clavibacter michiganensis* [Cm] CASJ009), and pathogenic (*L. xyli* subsp. *xyli* [Lxx] CTCB07, *L. xyli* subsp. *xyli* gdw1, *L. xyli* subsp. *cynodontis* [Lxc] DSM46306, *C. michiganensis* subsp. *sepedonicus* [Cms] ATCC33113, and *C. michiganensis* subsp. *michiganensis* [Cmm] UF1) bacteria were evaluated. Each genome sequence was compared with the protein reference sequence for each gene from *C. michiganensis* subsp. *michiganensis* NCPPB382, and percent amino acid similarity, E-value, and the query coverage was calculated using tblastn. Percent amino acid similarity (top value, bold), E-value (middle value), and query coverage (bottom value) is indicated in each cell, represented by a red-blue color scale based on percent amino acid similarity. Gray cells indicate that no significant similarity (NS) was identified within that genome.

share any significant amino acid similarity with any of the virulence genes, with the exception of SE134, which shared only 45% similarity with the *celA* gene. In addition, the three pathogenic *Leifsonia* strains share varying levels of amino acid similarity with the different virulence factors (Fig. 5). Our inclusion of commensal and pathogenic *Clavibacter* strains validated our approach, in that the pathogenic strains *C. michiganensis* subsp. *sependonicus* ATCC33113 and *C. michiganensis* subsp. *michiganensis* UF1 shared 50 to 100% amino acid similarity with each of the six virulence factors evaluated (Fig. 5).

Overall, this analysis provides evidence that C5G2, SE134, and M321 do not encode for the well-characterized plant-associated virulence factors investigated here. The difference in homology to these virulence factors is a clear distinguishing factor between beneficial or commensal and pathogenic *Leifsonia* strains. It should be noted that these virulence factors have not been functionally characterized in *Leifsonia* spp. Due to the lack of mechanistic understanding of *Leifsonia*–plant pathogenicity, the virulence factors described here provide reasonable candidates for future functional characterization within the genus.

The presence of genes involved in antioxidant production and ROS detoxification provide evidence of multiple putative mechanisms that allow plant-associated strains, including C5G2, to overcome a highly oxidative environment in the rhizosphere and ultimately colonize the plant host (Arenas et al. 2010; Cosgrove et al. 2007; Fontenelle et al. 2011; Hu et al. 1999; Inaoka et al. 1999; Jeong et al. 2000; Martínez-Granero et al. 2005; Si et al. 2019; Smirnova et al. 2015; Villalobos et al. 2019; Wiesner et al. 1988; Zuber 2009) (Fig. 4; Table 2). C5G2 was previously characterized for its ability to increase the growth of greenhouse ornamental crops after recovery from water stress. The genome analysis conducted here identified multiple genes potentially involved in bacterial osmotic stress tolerance and mechanisms involved in increasing plant water stress tolerance. Although the PGPR SE134 has not been evaluated for its ability to stimulate plant growth under water stress, these observations point to the potential for this PGPR to stimulate plant growth under different abiotic stresses.

After accounting for the orthologs shared between all genomes (PC 1), there were few orthologs shared between plant-associated or pathogenic *Leifsonia* spp. and the *Clavibacter* sp. tested (PC 3) (Fig. 3). This suggests globally independent mechanisms for plant association between these two genera but this remains to be experimentally tested. Our work did identify multiple OGCs across different PCs containing C5G2 that are related to carbohydrate metabolism, oxidative stress tolerance, bacterial osmotic stress tolerance, and response to nitric oxide (Fig. 4). Additionally, we identified OGCs for responses to antibiotics unique to each PC. Resistance to externally produced antibiotics from competing microorganisms in the plant rhizosphere can contribute to increased fitness (Lugtenberg and Kamilova 2009). *L. xyli* subsp. *xyli* is susceptible to ampicillin, tetracycline, and kanamycin (Brumbley et al. 2006), whereas our in vitro work showed that C5G2 is resistant to both ampicillin and kanamycin, providing another distinguishing characteristic between the biology of C5G2 and *L. xyli* subsp. *xyli*. Furthermore, C5G2 does not share any BGCs in common with the two pathogenic *L. xyli* subsp. *xyli* strains (Supplementary Table S5).

Beneficial *Leifsonia* spp., including C5G2, displayed an enrichment for carbon metabolism in the KEGG, OGC, and CAZyme analyses (Fig. 4; Supplementary Tables S1 and S3). This provides evidence of a robust central carbon metabolism, a likely mechanism for C5G2 to utilize plant-secreted carbohydrates and form a symbiotic relationship with its host. Enrichment of carbohydrate metabolism in the two PGPR strains, and not the pathogenic strains, is a

clear distinguishing factor in the biology of these organisms. The CAZyme profile showed a higher diversity of GHs for the beneficial and commensal strains compared with the pathogenic strains (Supplementary Table S3). Our in vitro carbon source utilization experiment corroborated our KEGG analysis findings, showing that C5G2 has a robust central metabolism. This difference could be due to the inability of nonpathogenic strains to adapt to several hosts and use difference carbon sources. Fastidious organisms have very specific nutritional requirements, often requiring a specific ecological niche to survive and proliferate. *L. xyli* subsp. *xyli* strains are fastidious organisms (Brumbley et al. 2004), and this suggests an adaptation to a more specific niche within the sugarcane host (Monteiro-Vitorello et al. 2004; Wang et al. 2016), whereas C5G2's ability to grow on a variety of sole carbon sources indicates a distinguishable, nonfastidious nature. The fastidious nature of SE134 is not documented. Additionally, our in vitro growth analysis of C5G2 shows that this strain reaches the exponential phase of growth significantly faster than documented *L. xyli* subsp. *xyli* pathogens (Brumbley et al. 2004). Finally, many CAZymes were identified exclusively in the beneficial or commensal strains, or even in C5G2 and not the pathogenic strains. This provides another point of distinction between the beneficial or commensal strains and the pathogenic ones.

These analyses begin to provide insight into potential mechanisms that these beneficial plant-associated *Leifsonia* spp. might be employing to increase plant growth and abiotic stress tolerance. Overall, this work provides a detailed genomic framework for analysis of *Leifsonia* spp. with multiple comparisons to other beneficial, commensal, and pathogenic strains. Although pathogenic species of *Leifsonia* have been well-characterized (Monteiro-Vitorello et al. 2004, 2013; Wang et al. 2016), work in previous years from different groups has provided evidence for the use of different species as plant-growth-promoting bacteria (Kang et al. 2017, 2014; Nordstedt and Jones 2020). The phylogenetic analysis provides clear evidence that *Leifsonia* sp. C5G2 is most closely related to other nonpathogenic *Leifsonia* spp. and is relatively distant to the known phytopathogenic strains in our analysis. Our other comparative analyses provide additional evidence of shared mechanisms between beneficial or commensal strains, with a lack of pathogenesis-related features. The genes identified within the genome of C5G2 involved in bacterial osmotic and oxidative stress, root surface adhesion, and the synthesis of tryptophan, folic acid, and plant osmoprotectants provide excellent candidates for future functional genetic analysis to further characterize C5G2's ability to stimulate plant growth under water-stress conditions. Although our previous work utilizing C5G2 as a PGPR focused on water stress, its potential utility to increase tolerance to other abiotic stresses should also be noted because plant responses to water and salt stress are closely related and many of the mechanisms overlap (Pandey et al. 2015; Zhu 2002). This work provides justification for future work within the genus *Leifsonia*, specifically with C5G2 to explore its utility as a plant growth promoter.

ACKNOWLEDGMENTS

We thank C. Taylor and Y. Xia at The Ohio State University for providing valuable input as internal reviewers of this manuscript.

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