Biological Control and Microbial Ecology Draft Genome Sequence Data of *Glutamicibacter* sp. FBE-19, a Bacterium Antagonistic to the Plant Pathogen *Erwinia tracheiphila*

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Abstract

Glutamicibacter sp. FBE-19 was isolated based on its strong antagonism to the cucurbit bacterial blight pathogen *Erwinia tracheiphila* on plates. Members of the *Glutamicibacter* genus can promote plant growth under saline conditions and antagonize fungi on plates via chitinolytic activity; however, their production of antibacterial compounds has not been examined. Here, we report the genome sequence of strain FBE-19. The genome is 3.85 Mbp with a G+C content of 60.1% and comprises 3,791 genes. Genes that may contribute to its antagonistic activity include genes for the secondary metabolites stenothricin, salinosporamide A, a second β -lactone compound, and a carotenoid. The *Glutamicibacter* sp. FBE-19 genome data may be a useful resource if this strain proves to be an effective biocontrol agent against *E. tracheiphila*.

Keywords

antagonistic, bacterial pathogens, biological control, bioinformatics, disease control and pest management, *Erwinia tracheiphila*, genome resource, genomics, *Glutamicibacter* sp.

Genome Announcement

Cucurbit bacterial wilt caused by *Erwinia tracheiphila* is an economically important disease in the Midwestern and Northeastern United States that is primarily controlled by managing the insects that transmit it, namely striped and spotted cucumber beetles (Rojas et al. 2015). Biological control of this pathogen would provide an alternative management approach, and one that would be desirable for integration into organic production systems. Thus far, there are few reports evaluating potential agents for biocontrol of this pathogen (Roberts et al. 2018).

In working with *E. tracheiphila* in the laboratory, a serendipitous contaminant was discovered that induced a large zone of inhibition on a lawn of the *E. tracheiphila* strain BHKY grown on King's B agar (Fig. 1). This zone of inhibition was larger than those produced by other bacterial strains, including *Paraburkholderia phytofirmans* (PSJN-1) and *Burkholderia pyrocinia* (55B1 and FP62), that had been examined as potential biocontrol agents (unpublished data). We designated this antagonistic strain FBE-19. Strain FBE-19 produced a similar-sized zone of inhibition in both the presence and absence of exogenous FeCl_3 (10 μM), indicating that the zone was not due to iron competition. We found that strain FBE-19 was highly antagonistic to all of the 42 *E. tracheiphila* strains examined, which included strains representing two distinct clades of *E. tracheiphila* (Shapiro et al. 2018), as well as to the closely related enteric bacterial species *Pantoea stewartii* (unpublished data).

Strain FBE-19 was identified by 16S ribosomal RNA (rRNA) sequencing as *Glutamicibacter* sp. This genus currently has six named species, and a BLASTn search of the NCBI database indicated that FBE-19 is most closely related to *Glutamicibacter halophytocola* strains DR408 and KLBMP-5180 (98% identity with each); however, when the 16S rRNA sequences of several

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Fig. 1. Antagonism and phylogeny of *Glutamicibacter* sp. strain FBE-19. **A**, Antagonistic effect of (1) *Glutamicibacter* sp. FBE-19 against *Erwinia tracheiphila* strain BHKY on King's B agar as compared with (2) a water control. **B**, Phylogenetic tree of selected *Glutamicibacter* strains based on ribosomal RNA proteins (constructed by Mauve2.4.0 and displayed by iTOL).

Glutamicibacter spp. were used for alignment, a phylogenetic tree analysis using MEGA7 indicated insufficient resolution to determine the *Glutamicibacter* sp.

The genus *Glutamicibacter* was reclassified in 2016 from *Arthrobacter* (Busse 2016). Bacteria of the genus *Glutamicibacter* have been found in a variety of environments, including from animals (Santos et al. 2020), cheese (Irlinger et al. 2005), and plants (Qin et al. 2018). *Glutamicibacter* isolates were shown to enhance the growth of the halophytic plant *Limonium sinense*, as well as tomato, rice, and soybean plants under saline or drought conditions (Feng et al. 2017; Ji et al. 2020; Nishu et al. 2019; Qin et al. 2018; Xiong et al. 2019). Although *G. nicotianae* was recently evaluated as an antagonist of quorum signal-mediated virulence of a soft rot pathogen (Vesuna and Nerurkar 2020), *Glutamicibacter* strains have not yet been examined for biocontrol activity mediated by the production of antimicrobial compounds. The genome sequence of *Glutamicibacter* sp. FBE-19 reported here may provide insights into traits contributing to the antagonism of *E. tracheiphila* or, more generally, to impacts of this genus on plant health.

Glutamicibacter sp. FBE-19 was grown on King's B medium for 2 days at 28°C and harvested for DNA extraction using the Gentra Puregene bacterial DNA extraction kit (Qiagen). The genomic DNA quality was assessed by visualization on a gel and was quantified using a Qubit Fluorometer. High-quality DNA (200 ng) was sequenced using the HiSeq3000 platform at the DNA Facility at Iowa State University, generating 100-bp paired-end reads. In total, 6,980,054 reads were obtained, and no sequences were flagged as poor quality when using FastQC (v0.11.9) (Andrews 2010) for quality analysis. The raw reads were trimmed with Trimmomatic (v0.39) using the parameters ILLUMINACLIP: Nexte, PE adapter:2:30:10:2: keep Both Reads, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, HEADCROP:14, and MINLEN:36. In total, 6,583,430 reads were used for the subsequent assembly. The genome was assembled by SPAdes v3.14.0 (Bankevich et al. 2012). The quality of the assembly was evaluated by QUAST v5.0.2 (Mikheenko et al. 2018). The genome was assembled into 22 contigs (>1,000 bp), with the largest contig being 798,790 bp and a total length of 3,846,453 bp. The N_{50} was 334,508 and L_{50} was 4. The G+C content of the genome is 60.1%.

The gene starts were predicted using the GeneMarkS program (version 4.17) (Besemer et al. 2001), which predicted a total of 3,665 genes. Of these, 1,833 were assigned to 23 Kyoto Encyclopedia of Genes and Genomes orthology groups using BlastKOALA (Kanehisa et al. 2016). The genome was annotated by RASTtk (Brettin et al. 2015) (Table 1), which predicted a total of 3,791 genes, with 1,903 on the positive strand and 1,888 on the negative strand, and the coding sequences composing 90.0% of the genome. The annotated genes included 3,704 protein-coding sequences (CDSs), with 2,487 (67%) with some functional prediction and 1,217 (34%) annotated as hypothetical proteins. The average CDS size was 929 bp, which varied from 114 bp, which encodes a thiol peroxidase, to 4,809 bp, which encodes a NAD-specific

Table 1. Genome information for the *Glutamicibacter* sp. FBE-19 strain sequenced in this study

Features	Results
Genome size (bp)	3,846,453
GC content (%)	60.1%
Number of total genes	3,791
Number of coding genes	3,704
Number of contigs	22 (>1,000 bp)
Genome coverage	181X
N ₅₀ (bp)	334,508
Maximum contig size (bp)	798,790
Ribosomal RNA operons	3
Transfer RNAs	65
Accession number	JACKQN000000000
Number of unannotated genes	1,217 (34%)

glutamate dehydrogenase. In addition, the RASTtk annotation identified 65 transfer RNAs and three complete rRNA operons. The genome includes 105 tandem repeats, as identified with the Tandem Repeats Finder (version 4.09) (Benson 1999). Using multilocus sequence typing of the 53 bacterial ribosomal protein subunits (Jolley et al. 2012), we identified strain FBE-19 as *G. halophytocola* (Fig. 1B).

Multiple genes were identified that may contribute to the antagonist activity of *Glutamicibacter* FBE-19 against other bacteria. We used antiSMASH (version 5.1.2) to detect gene clusters potentially capable of secondary metabolite synthesis. In particular, FEB-19 has biosynthetic gene clusters predicted to synthesize stenothricin, the β -lactone salinosporamide A, a second unidentified β -lactone compound, and a carotenoid. The FBE-19 genome is rich in proteins associated with iron scavenging, including the biosynthetic genes for desferrioxamine (also identified by antiSMASH), and several ABC-type siderophore transport systems, including for the siderophore petrobactin. The FBE-19 genome shows relatively little evidence of horizontal gene transfer, having only two clear toxin-antitoxin systems and four transposases. Finally, the FBE-19 genome has multiple features associated with osmoadaptation, including genes encoding an aquaporin and multiple transporters for osmoprotectant compounds, including OpuD for glycine betaine, BetT for choline, and three proline/glycine betaine ProP transporters.

Data availability. Glycerol stocks of *Glutamicibacter* sp. strain FBE-19 are available for academic purposes upon request. The genome sequence of FBE-19 is available in NCBI under the accession number JACKQN000000000, BioProject: PRJNA655947, and BioSample: SAMN15762367.

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