

Complete genome sequences of seven *Ralstonia solanacearum* phylotype IIB-4C/IIB-4E strains isolated from plantain trees in Ecuador

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ABSTRACT *Ralstonia solanacearum* has been causing an epidemic of Moko disease in Ecuador since early 2024. Here, we announce the publication of seven complete genome sequences from isolates belonging to the phylotype IIB sequevar IIB-4 and subclade IIB-4C/IIB-4E. Their genome megaplasmid is predicted to contain a class D beta-lactamase gene.

KEYWORDS *Ralstonia solanacearum*, moko disease, plantain disease

Ecuador faces an epidemic of Moko disease (1) since early 2024. We provide complete genomes of seven isolates from infected trees or surrounding soil.

We collected pseudostem tissue or surrounding soil between February and May 2024 (Table 1). A piece of tissue from the wounded area was retrieved, cleaned with water, surface-sterilized with 70% ethanol, cut with a scalpel, and gentle pressure applied to obtain the bacterial exudate for inoculation (2). Ten grams of soil (50 cm away and 15 cm deep) was mixed with sterile water (1:10), supernatant retrieved and diluted to 10⁻⁴ prior to inoculation (2). Processed samples were inoculated in SMSA plates and incubated for 72 h at 28°C aerobically (3). Presumptive *Ralstonia* colonies were isolated for genomic DNA extraction by restreaking in NASYE medium and incubated for 48–72 h at 28°C under aerobic conditions (4). DNA was extracted using the ZymoBIOMICS DNA Microprep Kit and quantified with Qubit 1X dsDNA HS assay. Libraries were prepared with the Native Barcoding Kit 24 V14 (ONT), without fragmentation or size selection, and sequenced in the GridION (R10.4.1 flow cells) with super-accurate basecalling (MinKNOW v23.11.7). Sequencing yielded 1.30M of demultiplexed raw reads (4.03 Kb N50 and 21 Q-score). Genomes were assembled with Flye v2.9.5 (5) and Raven v1.8.3 (6), processed with Tricycler v0.5.5 (7), and polished with Medaka v1.12.1. Default parameters were used for all software. Closed circular assemblies were returned by Tricycler for the three replicons in all isolates. Replicons were not reoriented. Completeness was assessed with Busco v5.8.2 (8) (data set: burkholderiales_odb10) and Quast v5.2.0 (9) (reference: [NZ_CP012943.1](#)). Taxonomy assignment and annotation were performed by NCBI with PGAP v6.8 (10). Phylogeny was inferred following the KBase narrative proposed previously (11), using the tool Insert Set of Genomes Into SpeciesTree v2.2.0 with the reference genome set RSSC_for_phylogenomics_v2023-11-11.

Seven closed circular genomes were obtained (66.5% GC content for all), each with a chromosome and two plasmids. All isolates were confirmed as *Ralstonia solanacearum* (ANI values to [GCA_015910705.1](#) in Table 1), and all scored 99% BUSCO completeness and 88% Quast similarity. Genome annotation identified a predicted class D beta-lactamase gene in the megaplasmid of each isolate. Phylogeny clustered all strains within phylotype IIB-4C/IIB-4E (Fig. 1).

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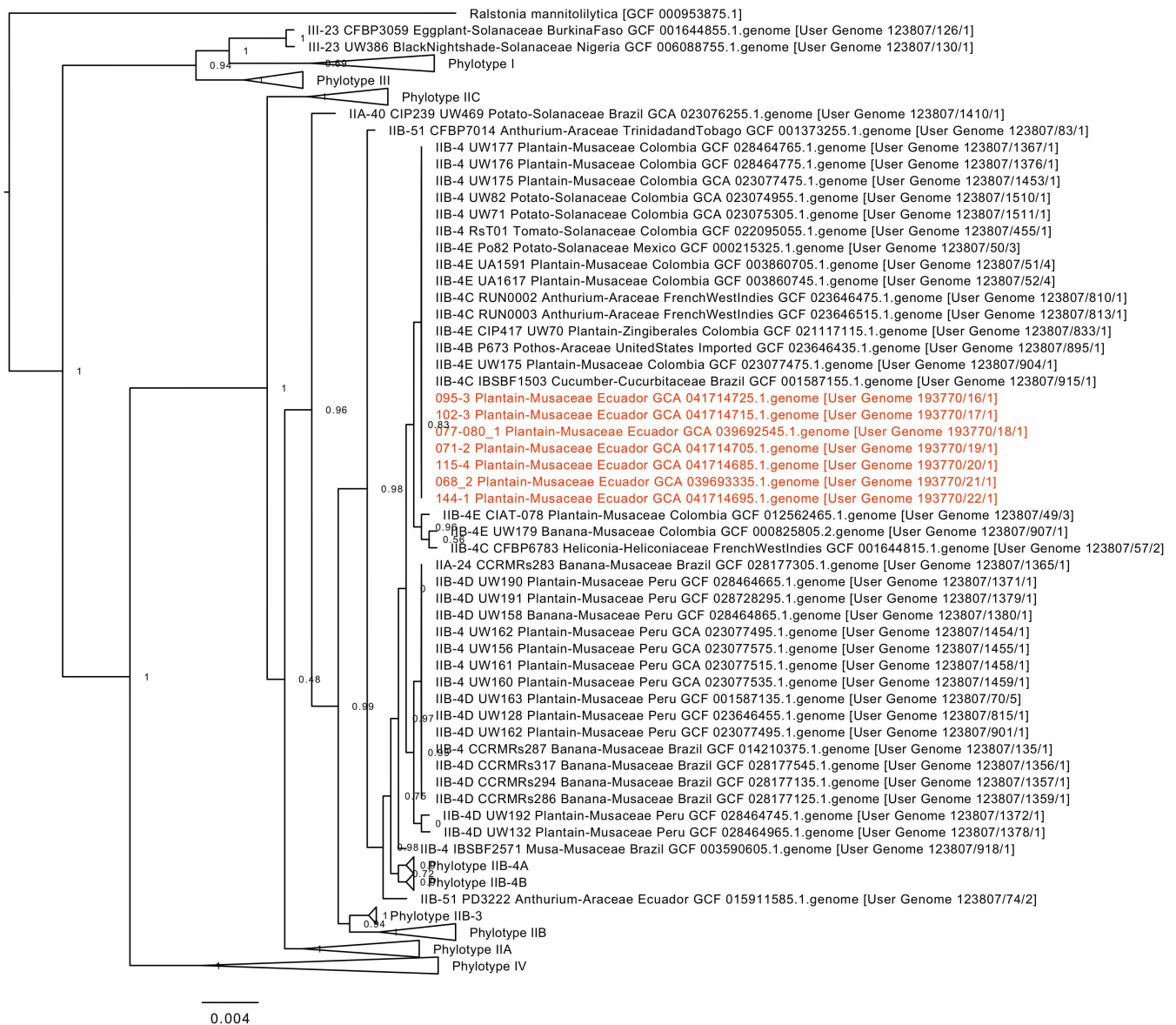


FIG 1 Phylogeny of isolates sequenced in this study (shown in red). Node labels are local support values computed with the KBase tool.

TABLE 1 Characteristics of isolates sequenced in this study^a

Isolate	Location	Date	SRA accession	Raw read number	Raw read N50 (bp)	NCBI accession	Size (bp) Chromosome Plasmid 1 Plasmid 2	ANI value (%)
068_2	Buena Fe, Los Ríos	08 February 2024	SRR33189762	80,886	6,323	ASM3969333v1	3503462 1951654 173870	96.14
077-080_1	El Empalme, Los Ríos	08 February 2024	SRR33189761	641,114	1,550	ASM3969254v1	3503477 1951655 173870	96.14
095-3	San Jacinto del Búa, Santo Domingo	20 May 2024	SRR33189767	190,622	1,986	ASM4171472v1	3500663 1941694 174509	96.15
102-3	San Jacinto del Búa, Santo Domingo	20 May 2024	SRR33189766	59,593	2,958	ASM4171471v1	3500655 1941693 174509	96.15
071-2	Arenillas, El Oro	20 May 2024	SRR33189765	82,526	2,693	ASM4171470v1	3500695 1950768 173863	96.14
144-1	La Unión, Los Ríos	20 May 2024	SRR33189764	128,087	4,544	ASM4171469v1	3500665 1944777 172529	96.15
115-4	Arenillas, El Oro	20 May 2024	SRR33189763	122,762	5,486	ASM4171468v1	3500755 1950766 173863	96.14

^aAll isolates were retrieved from pseudostems, except isolate 102-3 collected from soil. Only isolates 095-3 and 102-3 came from the same farm.

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Rommel Guevara, Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing | Teresa Guerrero, Investigation, Methodology, Writing – original draft | Nicol Barriga, Investigation, Methodology, Writing – review and editing | Erika Muñoz, Investigation, Methodology, Software, Writing – review and editing | Denisse Benítez, Formal analysis, Investigation, Methodology, Software | Paúl Cárdenas, Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

Genome sequences and raw read data are deposited in NCBI (accession numbers in Table 1).

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