Identification of phylotype II sequevar 1 (race 3 biovar 2) as the causal *Ralstonia solanacearum* subgroup from the 2020 US geranium introduction

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Abstract
Ralstonia solanacearum phylotype II sequevar 1 (*Rs*II-1, formerly race 3 biovar 2) causes tomato bacterial wilt, potato brown rot, and Southern wilt of geranium. Strains in *Rs*II-1 cause wilting in potato and tomato at cooler temperatures than tropical lowland *R. solanacearum* strains. Although periodically introduced, *Rs*II-1 has not established in the United States. This pathogen is of quarantine concern and listed as a Federal Select Agent. We report a rapidly sequenced (<2 days) draft genome of UW848, a *Rs*II-1 isolate introduced to the United States in geranium cuttings in spring 2020. UW848 belongs to the near-clonal cluster of *Rs*II-1 global pandemic strains.

Genome announcement

Ralstonia solanacearum phylotype II sequevar 1 (*Rs*II-1, known historically and for regulatory purposes as race 3 biovar 2) causes vascular wilt diseases of diverse ornamental and solanaceaous crops, most notably potato brown rot. Brown rot is among the most serious threats to tropical highland potato growers worldwide. *R. solanacearum* II-1 can be disseminated by latently infected potato seed tubers and geranium cuttings (Swanson et al. 2007; Scherf et al. 2010). Strains in this subgroup, which originated in the Andes but has been globally distributed, induce wilting in plants at cooler temperatures than *R. solanacearum* tropical lowland strains (Allen, C. et al. 2001; Milling et al. 2009). Although the pathogen has been accidentally introduced previously to North America in geranium cuttings, to date it has not become established there. The *Rs*II-1 subgroup is a quarantine pathogen and a U.S. Select Agent (Lambert 2002). Following an accidental introduction of *Rs*II-1 in geranium in spring 2020, we used rapid genome sequencing to identify and characterize the responsible *R. solanacearum* strain.

Bacteria with typical *R. solanacearum* colony morphology were isolated from four wilted geranium plants (cv. Fantasia ‘Pink Flare’). All were identified as *Ralstonia* species based on immunostrip tests (Agdia, Inc. Elkhart IN) and were determined to be *Rs*II-1 using the phylotype multiplex PCR and the 630/631 primer pair (Tran et al. 2015; Fegan and Prior 2005). Genomic DNA was extracted from four isolates using Epicentre MasterPure genomic DNA kit and sequenced on an iSeq 100 Illumina benchtop sequencer with the Nextera DNA Flex Library Prep protocol kit and Nextera DNA CD indexes. 150bp paired-end reads were assembled using SPAdes version 3.13 with k-mer sizes of 55,75,95 (Nurk et al. 2013). Whole genomes were compared using average nucleotide identity (ANI), with reference genomes from all four phylotypes of the *R. solanacearum* species complex (Rodriguez-R and Konstantinidis 2016; Remenant et al. 2010). Genomes of the four geranium isolates, which were almost identical to each other, had 99.98% ANI to the well studied *Rs*II-1 strain UW551, isolated from geranium in 1999 (Hayes et al. 2017; Williamson et al. 2002).

On average, each genome assembly had 48x coverage and 150 SNPs compared to the UW551 genome (Li et al. 2009; Li and Durbin 2009). One selected genome assembly, corresponding to strain UW848, has a GC content of 66.7%, with 5,353,250 bp in 160 contigs. Functional annotation of the contigs was done using Prokka v1.13.3, and secreted proteins were predicted based on SignalP algorithm as part of Prokka analysis (Seemann 2014; Petersen et al. 2011). UW848 genome encodes 4524 genes, of which 490 are predicted to encode secreted proteins. The temperature-responsive gene cluster *lecM-aidA-aidC-solR-solI*, required for full virulence, was complete, and the encoded proteins had 100% amino acid identity with UW551 (Meng et al. 2015). Overall, the genome of *Rs*II-1 strain UW848 provides a resource for immediate pathogen identification and tracking and for future studies to better understand the molecular epidemiology and biology of this highly regulated quarantine pathogen.

The genome of UW848 is deposited at GenBank under BioSample and Bioproject accession number SAMN15102643 and PRJNA637338, respectively, and was uploaded to LINbase (Tian et al. 2020) where it was assigned the LIN $14_A1_B0_C0_D0_E3_F0_G0_H0_I0_J1_K0_L0_M0_N0_O0_P0_Q3_R0_S0_T$.

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