A Meta-analysis of the known Global Distribution and Host Range of the *Ralstonia* Species Complex

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Abstract

The *Ralstonia* species complex is a genetically diverse group of plant wilt pathogens. *Ralstonia* strains are classified by a "phylotype-sequevar" phylogenetic system. Since the development of the phylotype-sequevar system, over one hundred papers have described the genetic diversity of *Ralstonia* strains isolated from agriculturally important crops, ornamental plants, and plants in natural ecosystems. Our goal is to create a database that contains the reported global distribution and host range of *Ralstonia* sequevars. In this second release, we have catalogued information from 73 manuscripts that report one or more *Ralstonia* strain isolated from 88 geographic regions. The database is hosted as a GitHub repository (https://github.com/lowepowerlab/Ralstonia_Global_Diversity) that will be updated regularly.

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Introduction

Bacterial pathogens in the *Ralstonia* species complex are xylem pathogens on a broad range of agricultural and natural plant hosts. *Ralstonia* strains clog plant xylem vessels, leading plant hosts to wilt [1]. Historically, *Ralstonia* strains were classified based on carbon utilization patterns ("Biovar") and host range ("Race"). However, DNA sequence-based taxonomies more accurately reflect the evolutionary trajectories of *Ralstonia* (Fig 1).

Currently, the *Ralstonia* species complex is classified into three species: *R. solanacearum*, *R. pseudosolanacearum*, and *R. syzygii*. Strains are also classified into a phylotype system, which overlaps with the species boundaries. All *R. solanacearum* strains are within phylotype II, but phylotype II is divided into IIA and IIB groups. *R. pseudosolanacearum* strains are either in phylotype I or phylotype III. *R. syzygii* strains are in phylotype IV. Strains are further sub-classified into sequence variants, or "sequevars", based on the DNA sequence of *egl* and other reference genes. The phylotype-sequevar system developed and described by Prior and Fegan [2]. The separation of *Ralstonia* into three species was first proposed by [3], formalized by [4], and reinforced by [5]. Hundreds of papers have used the phylotype-sequevar system to describe the genetic diversity of *Ralstonia* isolates around the world.

The *Ralstonia* community typically states that *Ralstonia* strains infect over 250 plant species in over 50 botanical families. Is that an under-estimation? Our goal is to perform a meta-analysis that documents the known host range and global distribution of each sequevar in the *Ralstonia* species complex. We intend to update this preprint and the associated GitHub repository at regular intervals as we populate the database.

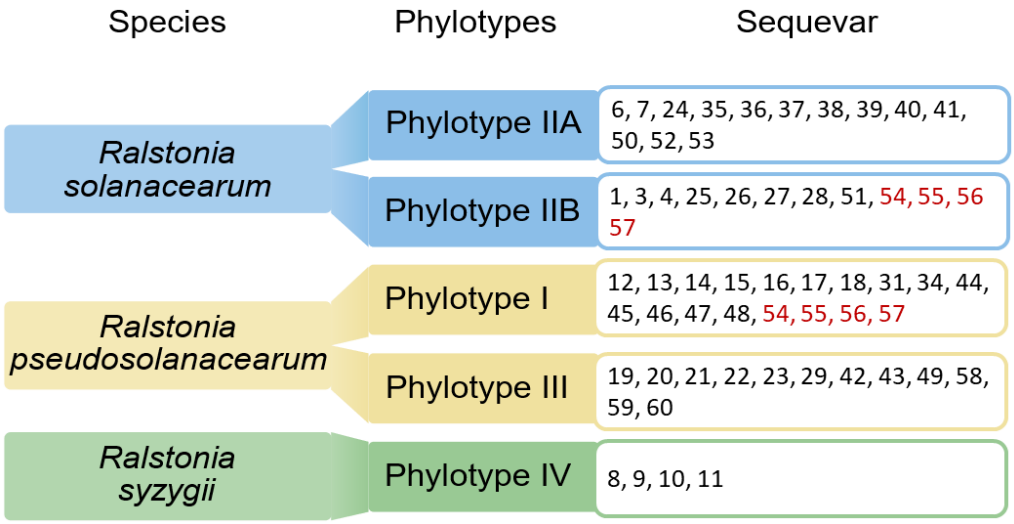

Figure 1. Taxonomic classification of the *Ralstonia* species complex at the species, phylotype and sequevar levels. Sequevars highlighted in red are assigned to strains in conflicting phylotypes.

Methods

Article Selection Criteria and Search Strategy:

To catalogue sequevar and geographic distributions for newly described *Ralstonia* strains, we performed a secondary analysis of the paper that cite "How Complex is the *Ralstonia solanacearum* species complex?" by Fegan and Prior 2005 [2]. Using Google Scholar, we identified the papers that both

cite Fegan and Prior, 2005 and include the word "sequevar". The most recent papers are prioritized for addition to the database in each release with the eventual goal to catalog all papers.

To be included in the database, the articles must have used the phylotype and/or phylotype-sequevar system to characterize the strains. Studies that used biovar and race identification methods without using a phylogenetic method were not included. Papers focusing on molecular biology of single or well-documented strains were also not included because they did not report information about new isolates.

Database access:

The full dataset is stored in a GitHub repository. We anticipate formal updates to the database once to twice per year. https://github.com/lowepowerlab/Ralstonia_Global_Diversity

Results and Discussion

We compiled 3395 strains from 73 papers published between 2012 and 2020 [4–77] , including the common reference strains for *Ralstonia* sequevar studies. These strains represent over 64 sequevars isolated from 88 countries or territories (Table 1).

The full dataset is included as Table S1 and is hosted in the GitHub repository. For each strain, we recorded taxonomy (phylotype and sequevar), host (specific name and the host plant's taxonomic Family and Order), isolation year, isolation location, NCBI accessions (genome or partial sequences of *egl* and housekeeping genes like *mutS* and/or *rplB* genes) and the citation.

Table 1: Summary of the Sequevar database

Database Release Date	# Papers	Paper published range	# Strains	# Sequevars	# Countries or Territories	# Host plants
2020/07/03	35	2017-2020	1625	57	50	56
2021/03/23	73	2012-2020	3395	over 64[a]	88	124

[a]*As of this release, we have not investigated the sequevar assignment of strains classified into "unknown" or "new" sequevars.*

We investigated which sequevars have been isolated from multiple locations (Table 2). We classified the geographic location to either country level or to the regional level for island territories. Even with this incomplete dataset, several sequevars were identified in multiple locations. As expected [64], the IIB-1 sequevar is the most widely distributed group of *Ralstonia*. IIB-1 *Ralstonia* cause brown rot of potato and can be latently disseminated in both potato tubers and ornamental Geranium sp. [78]. This group of strains is under strict quarantine worldwide and are classified as Select Agent pathogens in the United States. An additional three phylotype II sequevars (IIB-4, IIA-6, and IIA-7 and six phylotype I sequevars (I-13, I-14, I-15, I-17, I-18, and I-31) were isolated from at least eight geographic areas.

Table 2: Widely distributed sequevars

sequevar	# countries or territories
IIB-1	41
IIB-4	12
IIA-6	9

IIA-7	8
I-13	9
I-14	10
I-15	8
I-17	8
I-18	11
I-31	10

We investigated which sequevars have been isolated from diverse host plants (Table 3). We classified the host to the species and family level, based on the information presented in the primary literature. Most of the sequevars that are widely distributed have broad host ranges. Ten sequevars have been isolated from at least 10 plant species in 5 to 17 botanical families.

Table 3: Broad host range sequevars

sequevar	# plant species	# plant families
IIB-1	15	5
IIB-4	20	7
I-13	18	12
I-14	27	17
I-15	26	14
I-17	21	10
I-18	13	8
I-31	13	5
I-44	17	13
I-48	10	5

Conclusion

Bacterial wilt pathogens in the *Ralstonia* species complex are high impact global pathogens. We created a strain database that we will regularly update to document the distribution and host range of *Ralstonia*. The second release of the database contains data from 73 papers.

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