



# Exploring the genetic diversity of recent *Ralstonia pseudosolanacearum* phylotype I findings in Europe

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**Abstract** The increasing prevalence of *Ralstonia pseudosolanacearum* phylotype I (phy I) in agricultural regions across Europe highlights the need for heightened awareness and preventive measures. Recent detections in multiple European countries span a range of agricultural systems, host plants, and natural aquatic environments. These findings have been linked either to infected plant material at import (interceptions) or to plants cultivated in specific

European regions, often after importation of infected plant material. To investigate the genetic diversity of these emerging populations, we analysed whole-genome sequence (WGS) data from a collection of 62 *R. pseudosolanacearum* phy I isolates, including those recently obtained in various European countries, using average nucleotide analysis (ANI) and core genome multilocus sequence typing (cgMLST). ANI analysis revealed two distinct major clades: the

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"rose clade", comprising isolates from rose, potato, surface water, and bitterweet, most closely matching with sequevar 33, and the "major ginger clade", including isolates from members of the Zingiberaceae family, with the best match to sequevar 30. Additionally, several minor clades of Zingiberaceae isolates and a "tomato clade" (most closely related to sequevar 18) were identified. Pathogenicity assays with high inoculation titers were performed on tomato to assess the biological relevance of the observed genetic diversity. Our findings suggest multiple independent introduction events of virulent *R. pseudosolanacearum* phy I populations into Europe between 2015 and 2024. Given the genetic variability of the introduced bacterial isolates, the pathogen's ability to systemically infect a broad range of host plants which can differ per isolate, and its persistence in diverse agricultural and environmental niches, the implementation of stringent import control measures for plant material entering the European Union is strongly recommended.

**Keywords** *Ralstonia pseudosolanacearum* · Surface water · Zingiberaceae · Solanaceae · Rosaceae

*Ralstonia pseudosolanacearum* phylotype I (phy I), the causal agent of bacterial wilt, is a recognized invasive plant pathogen in both agricultural and natural ecosystems (Vaillau & Genin, 2023). It is listed as a quarantine organism by the European Union (EU). Following the taxonomic revision of the *Ralstonia solanacearum* species complex (RSSC), *R. pseudosolanacearum* (phy I and III) was designated as one of the three described bacterial species within the RSSC, alongside *Ralstonia solanacearum* (phy II) and *Ralstonia syzygii* (phy IV) (Prior et al., 2016; Safni et al., 2014). *R. pseudosolanacearum* phy I has a remarkably broad host range, capable of infecting over 300 plant species belonging to more than 40 plant families. These include major crops, such as potato and tomato, as well as a wide variety of ornamentals (EFSA Panel on Plant Health (EFSA PLH Panel) et al., 2019; Genin & Denny, 2012; Paudel et al., 2020; Savary et al., 2019). Members of the RSSC infect host plants via their roots, primarily through wounds, and rapidly colonize the xylem vessels, spreading systemically through the plant

(Genin, 2010). Similar to *R. solanacearum* phy II, *R. pseudosolanacearum* phy I can survive and multiply in aquatic weeds and disperse through surface water (Vogelaar et al., 2023), making eradication efforts challenging or even impossible (Álvarez et al., 2010; Elphinstone, 2005). The international trade of plants has contributed to the spread of members of the RSSC globally, facilitated in part by the long latency period before visible symptoms of the disease develop and appear in infected plants (Elphinstone, 2005). Therefore, implementation of stringent import control measures for plant material entering the European Union is strongly recommended.

Several studies have demonstrated substantial genetic diversity within the RSSC (Subedi et al., 2024; Yahiaoui et al., 2017) with individual *R. pseudosolanacearum* phy I isolates differing in terms of virulence and host range (Overeem et al., 2023; Sharma et al., 2022). Although *R. pseudosolanacearum* phy I remains virulent under cooler temperatures (Bocsanczy et al., 2012, 2017; Dewberry et al., 2024), it primarily thrives in warm, humid conditions typical of tropical climates, where bacterial population densities can reach  $10^8$  colony-forming units (CFU) per gram of plant tissue during late stages of the disease (Álvarez et al., 2010; Rivera-Zuluaga et al., 2023). Rising global temperatures due to climate change are likely to increase the risk of *R. pseudosolanacearum* phy I outbreaks in temperate regions. The International Plant Protection Convention (IPPC) has recently emphasized that climate shifts, particularly temperature changes, may significantly impact the distribution, severity, and management of plant pathogens (IPPC Secretariat, 2022).

Over the past decade, numerous studies have demonstrated the expansion of the geographical distribution of *R. pseudosolanacearum* phy I, which has spread far beyond its center of origin in Asia (Lowe-Power et al., 2024). Until recently, this bacterium had not been detected in outdoor agricultural systems or natural aquatic environments in Europe, and it had only been sporadically reported in restricted greenhouse cultivation systems. Europe has now seen several outbreaks of *R. pseudosolanacearum* phy I in cultivated crops, including roses, tomato (*Solanum lycopersicum*) and ginger (*Zingiber officinale*), either with or without symptoms (Table 1). Symptoms were similar, including wilting, leaf yellowing, and brown vascular discoloration (Fig. 1). The findings of *R.*

**Table 1** Findings of *Ralstonia pseudosolanacearum* phy I in Europe in recent years

Isolation source	Year	Country	Import/cultivated material	Presence of symptoms	Reference
<i>Rosa</i> sp.	2015–2018	Netherlands	Cultivated material	Symptomatic & asymptomatic	EPPO 2015/182*, 2020/040, 2017/017, Tjou-Tam-Sin et al., 2017a, 2017b
<i>Rosa</i> sp.	2015	Belgium	Import	Symptomatic	EPPO 2016/039
<i>Rosa</i> sp.	2016, 2022	Poland	Cultivated material	Symptomatic & asymptomatic	EPPO 2017/018, 2022/170
<i>Rosa</i> sp.	2021	Greece	Cultivated material	Symptomatic	EPPO 2024/088, Holeva et al., 2024
Irrigation water (from rose)	2015–2018	Netherlands			EPPO 2020/040
<i>Solanum lycopersicum</i>	2020–2022	Italy	Cultivated material	Symptomatic	EPPO 2022/194
<i>Solanum lycopersicum</i>	2021	Germany	Cultivated material	Asymptomatic	EPPO 2021/140
<i>Cucumis sativus</i>	2022	Germany	Cultivated material	Asymptomatic	EPPO 2022/195
<i>Zingiber officinale</i>	2021–2023	Germany	Cultivated material %	Symptomatic & asymptomatic	EPPO 2021/140, 2023/092
<i>Zingiber officinale</i>	2022	Germany	Cultivated material	Symptomatic	EPPO 2022/195
<i>Zingiber officinale</i>	2022–2023	Switzerland	Cultivated material %	Symptomatic & asymptomatic	EPPO 2023/187
<i>Zingiber officinale</i>	2023	Slovenia	Cultivated material %	Symptomatic	EPPO 2023/234, this paper
<i>Zingiber officinale</i>	2024, 2025	Netherlands	Import	Symptomatic & asymptomatic rhizomes	This paper
<i>Curcuma longa</i>	2021–2023	Germany	Import	Symptomatic rhizomes	This paper
<i>Solanum tuberosum</i>	2017 <sup>&amp;</sup>	Hungary	Cultivated material	Asymptomatic	This paper
<i>Solanum dulcamara</i>	2021–2023	Netherlands		Symptomatic & asymptomatic	EPPO 2023/254, Vogelhaar et al., 2023
Surface water	2022	Hungary			EPPO 2022/242
Surface water	2020–2023	Netherlands			EPPO 2021/179, 2023/165, 2023/254

\* EPPO reporting ID in EPPO global database (EPPO, 2025)

<sup>&</sup> Identified in 2023 after re-analysis of older RSSC findings

<sup>%</sup> 1st year of cultivation after import of rhizomes

*pseudosolanacearum* phy I in tomato and cucumber (*Cucumis sativus*) in Germany (Table 1) were linked to ginger, as these plants were grown in the same greenhouse. In response to the finding of *R. pseudosolanacearum* phy I in cultivated ginger, the EU implemented stricter import checks for ginger and curcuma rhizomes (European Commission, 2024). These measures led to the interception of *R. pseudosolanacearum* phy I in several consignments of rhizomes, identifying this as an introduction pathway into the EU. Beyond cultivated plants, *R. pseudosolanacearum* phy I has been found in surface water in The Netherlands and Hungary, as well as in the weed

bittersweet (*Solanum dulcamara*) (Table 1), indicating the pathogen's potential to establish in the EU. A noteworthy finding is the retrospective identification of *R. pseudosolanacearum* phy I from asymptomatic potato samples from Hungary in 2017. These samples were initially broadly classified as RSSC and were re-analyzed after the finding of the pathogen in surface water, leading to the first confirmed report of *R. pseudosolanacearum* phy I in potato on the European continent.

For the recent *R. pseudosolanacearum* phy I findings, the diagnostic procedure described in EU 2022/1193 (European Commission, 2022) was



**Fig. 1** *R. pseudosolanacearum* phy I symptoms observed in naturally-infected plant material in European countries. (A): Black necrosis of pruned branches and purulent discharge of creamy white slime on cut wounds in the stems of ornamental rose plants in the Netherlands. Necrosis and intense brown discoloration of the stems at the lower part of the rose plants, just above the ground, in Poland. Severe wilting of young rose shoots in the Netherlands; (B) Collapsed tomato plants during fruit enlargement in fields in Italy. Severe wilting, leaf yellowing, browning of the shoots, stem collapse, and desiccation of infected tomato plants; (C) Ginger plants under greenhouse and foil cultivation in Germany showed leaf yellowing, brown-

ing of the shoots, wilting, and desiccation. Internal glassy discoloration and light brownish of a ginger rhizome upon cutting. Structural grey discoloration in parts of the endodermis in a curcuma rhizome upon cutting; (D) Ginger plants cultivated in a foil tunnel in Switzerland showed leaf yellowing, browning of the shoots, wilting, and desiccation; (E) Brown discoloration with discharge of creamy white bacterial slime upon cutting of a ginger rhizome infected with *R. pseudosolanacearum* in the Netherlands; (F) Stem segments of bitterweet plants in the Netherlands showing discoloration of the vascular tissue. All images are shown on a different scale. Panel B images were kindly provided by C. Delvago

followed. *R. pseudosolanacearum* was isolated from small pieces of vascular tissue of symptomatic or

asymptomatic plants, specifically stems or petioles of rose and tomato plants or roots of rose, potato tubers

or ginger and curcuma rhizomes. For ginger, approximately 20 rhizomes were visually inspected for internal vascular symptoms. Each rhizome was cut longitudinally and vascular tissue from symptomatic plant material was immersed in (approximately 5 mL) 0.01 M phosphate-buffered saline (PBS) and incubated at room temperature (RT) for 30 min with agitation (100 rpm). After incubation, 50  $\mu$ L of the extract was plated onto semi-selective mSMSA plates using dilution plating (Elphinstone et al., 1996).

Vascular tissue from asymptomatic plant material was first macerated and then mixed with (approximately 10 mL) 0.05M phosphate buffer (PB) followed by incubation under the same conditions. In some cases, tissue debris was removed by filtration and/or low-speed centrifugation. Depending on the buffer volume, extracts were sometimes concentrated by centrifugation (e.g. 15 min at 7,000 g), after which the supernatant was discarded, and the pellet resuspended in 1.5 mL 0.01M PB. Subsequently, 50  $\mu$ L was plated onto semi-selective mSMSA plates using dilution plating (Elphinstone et al., 1996).

For surface water samples, two 50 mL aliquots were each concentrated by high speed centrifugation (e.g. 15 min at 7,000 g), resuspended in 0.5 mL 0.1M or 0.01M PB and plated using 100  $\mu$ L aliquots on semi-selective mSMSA medium (Elphinstone et al., 1996). Incubation of the mSMSA plates was performed for up to six days at 28°C. Colonies with typical *Ralstonia* morphology on mSMSA medium (fluidal purplish colour) were purified after incubation on nutrient agar (NA) or yeast peptone glucose agar (YPGA) media (Lelliott & Stead, 1987) for two to three days at 28°C as described previously (Bergsma-Vlami et al., 2018). DNA was extracted from pure cultures by heating at 95°C for 15 min or using commercial kits (DNeasy Blood and Tissue Kit (QIAGEN, Netherlands) or, for strain NIB Z 3685, the Genra Puregene Yeast/Bact. Kit (QIAGEN, according to the protocol for Gram-negative bacteria) and identified by TaqMan® real-time PCR (Vreeburg et al., 2016, 2018; Weller et al., 2000). Alternatively, a conventional PCR (Patrik et al., 2002) or an immunofluorescence test (IF test; LOEWE 07356/01 antiserum) was used for identification purposes. The determination of the phylotype was performed by multiplex conventional PCR (Fegan & Prior, 2005), DNA barcoding on the partial endoglucanase (*egl*) and *mutS* genes (Wicker et al., 2007), or

by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (van de Bilt et al., 2018). Sequevar assignment was based on the *egl* gene sequence used for RSSC phylogenetic inference, following the previously described methodology (Cellier et al., 2023b).

Whole-genome sequencing (WGS) was performed on multiple platforms: strain PD 7123 was sequenced on the PromethION P2 Solo (Oxford Nanopore Technologies (ONT), UK), while other isolates were sequenced on the NovaSeq 6000 (either at GenomeScan in Leiden, Netherlands or at Eurofins Genomics, Ebersberg, Germany), the PacBio Sequel II system (NIB Z 3685 at Novogene GmbH, Munich, Germany), or the NextSeq 2000 (Illumina, USA). ONT sequencing was performed using the Native Barcoding Kit 24 V14 following the gDNA protocol. For the Novaseq 6000, libraries were prepared using the NEBNext® Ultra II DNA Library Prep kit, with fragmentation performed on a Covaris ME220, followed by adapter ligation and PCR amplification. Quality and yield were assessed with the Fragment Analyzer (Agilent Technologies, USA) or Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA). For PacBio sequencing, HiFi SMRTbell libraries were prepared and sequenced using the PacBio Sequel II system (Pacific Biosciences, USA). PacBio raw reads were processed to generate circular consensus sequences (CCS) using pbccs v6.4.0 (Pacific Biosciences). For the NextSeq 2000, libraries were prepared using the Illumina DNA Prep (M) Tagmentation kit with Illumina Unique Dual Indexes. Five amplification cycles were performed, with quality control via a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) and a D1000 ScreenTape on the TapeStation 4200 (Agilent Technologies, USA). *Ralstonia* libraries were equimolarly pooled and 150PE sequencing was performed with a 300-cycle P1 cartridge and flow cell combination. Read assembly was performed as follows: 1) Illumina reads were assembled using SPAdes v3.14 in shovill 1.1.0 (GBBC 3080) or SKESA v2.5.0 (for all other isolates) (Prjibelski et al., 2020; Souvorov et al., 2018). 2) PacBio reads were assembled using Flye v2.8.1-b1676 (Kolmogorov et al., 2019). 3) ONT reads were assembled using Autocycler v0.2.0 with Canu v2.2, Flye v2.9.5, Miniasm v0.3, NECAT v0.0.1, NextDenovo v2.5.2, and Raven v1.8.3, followed by sequence correction with Medaka v2.0.1 (Wick et al., 2025). Raw read files, assemblies and

annotations have been deposited under DDBJ/ENA/NCBI BioProjects PRJNA1122974 (GBBC 3080), PRJNA1175583 (NIB Z 3685), and PRJNA1227534 and PRJNA663270 (all other isolates, Table 2).

Average Nucleotide Identity (ANI) analysis was performed using PYANI v0.2.12 (ANIm method) (Pritchard et al., 2016). The output was processed in R v4.4.0 using ape v5.8 and the phylogenetic tree was generated using ggtree v3.12.0 and tidyverse v2.0.0 (Paradis & Schliep, 2019; Wickham et al., 2019; Yu et al., 2017). Whole genome Multi Locus Sequence Typing (wgMLST) was performed using ChewB-BACA v3.3.5 (M. Silva et al., 2018), using a training file created with isolate PD 7123 for the "rose clade" and GMI1000 for the "major ginger clade" (Salanoubat et al., 2002). A wgMLST scheme was generated for the "rose" and "major ginger" clades. Subsequently, the generated scheme was used to perform allele calling on the two distinct clades. Finally, after removing paralogous genes, a cgMLST was determined, of which the allele call on the number of loci present in all genomes was used to generate a minimum spanning tree (MST) in R v4.4.0, with packages ape v5.8, ggnetwork v0.5.13, igraph v2.03, and tidyverse v2.0.0 (Briatte, 2024; Csárdi et al., 2024; Paradis & Schliep, 2019; Wickham et al., 2019). All R scripts are available at <https://github.com/NPPO-NL/Rpseudo-europe>.

The ANI-based phylogenetic tree reveals two major clades: the "rose clade", comprising isolates from rose, potato, surface water, and bittersweet, and the "major ginger clade", comprising isolates from Zingiberaceae family members (Fig. 2A). Within the "rose clade", all *R. pseudosolanacearum* phy I isolates were most closely associated with sequevar 33 (Table 2). These included the recent findings in rose, potato, surface water, and bittersweet, as well as the previously described Indian isolate Rs-09–161 from eggplant (Ramesh et al., 2014). ANI scores within this clade were consistently high (> 99.97%). A distinct sub-lineage within the "rose clade" was also associated with sequevar 33 and consisted of four *R. pseudosolanacearum* phy I isolates obtained from imported plant material intercepted in the Netherlands between 1988 and 2006, including: anthurium (PD 1255-Réunion Island, France and PD 5415-Ghana), potato (PD 3634-Kenya) and tomato (PD 3597-Kenya) (Fig. 2A; Table 2). Despite sharing the same sequevar designation (sequevar 33), these

isolates formed a genetically distinct lineage (ANI  $\approx$  99.3%) within the ANI tree (Fig. 2A), highlighting the limitations of sequevar classification in fully capturing phylogenetic relationships. The "major ginger clade" included nineteen *R. pseudosolanacearum* phy I isolates from Zingiberaceae hosts collected between 1993 and 2024. These isolates were associated with sequevar 30 (Table 2). However, several recently identified *R. pseudosolanacearum* phy I isolates in Germany from imported curcuma (AGQB#45, AGQB#119 and AGQB#48, all sequevar 17) and ginger (AGQB#125, sequevar 14) are outside the "major ginger clade" not clustering together. This demonstrates considerable genetic diversity among isolates from these hosts (Fig. 2A). An additional small clade was identified based on two *R. pseudosolanacearum* phy I isolates recovered from outdoor tomato cultivation in Italy (CREA-DC 2104 & 2105, sequevar 18). Genetically distinct from the "rose clade" and the "major ginger clade" these isolates appear to represent an independent introduction event. Their closest known relative in this analysis is Rs-10–244, which was isolated from *Capsicum* in India in 2010 (Ramesh et al., 2014). Additional comparison with publicly available *Ralstonia* genomes revealed a high degree of similarity with strains SM732 and SM734. These strains were both isolated in Bangladesh in 2012 from *Solanum melongena* plants grafted on *Solanum sisymbriifolium* rootstock and *Solanum virginianum*, respectively (data not shown) (Subedi et al., 2024).

To further explore intra-clade diversity, cgMLST analyses was performed using ChewBBACA. The core-genome, defined as the set of protein-coding genes shared by all isolates, consisted of 4,405 genes for the "rose clade" and 3,795 genes for the "major ginger clade". Despite having similar overall genome sizes, the smaller core-genome of the "major ginger clade" indicates a higher level of genomic variability among its isolates.

Minimum spanning tree (MST) analysis based on cgMLST revealed very limited variation among the "rose clade" isolates (Fig. 2B). All isolates were considered clonal due to SNPs in 0–3 core genes, except for Rs-09–161 (25) (Ramesh et al., 2014), which showed minor divergence of 73 core genes with SNPs compared to PD 8114 (3) and PD 7901 (4). In contrast, the "major ginger clade" exhibited three distinct clonal lineages and two distinct singletons (Fig. 2C):

**Table 2** *R. pseudosolanacearum* phy I isolates used in this study

Isolate	Isolated from	Country of origin	Country of isolation	Year	Best matching sequevar	Assembly number
Rs-10-244	Capsicum sp.	India	India	2010	48	GCA_000671315.1
PD 4190	Momordica charantia	Philippines	Netherlands	2001	45	GCA_048285315.1
1449-11/2017	Solanum tuberosum	Hungary	Hungary	2017	33	GCA_048287215.1
1451-11/2017	Solanum tuberosum	Hungary	Hungary	2017	33	GCA_048287255.1
1452-11/2017	Solanum tuberosum	Hungary	Hungary	2017	33	GCA_048287175.1
1453-11/2017	Solanum tuberosum	Hungary	Hungary	2017	33	GCA_048287195.1
1454-11/2017	Solanum tuberosum	Hungary	Hungary	2017	33	GCA_048287315.1
1455-11/2017	Solanum tuberosum	Hungary	Hungary	2017	33	GCA_048287295.1
508-11/2022	Surface water	Hungary	Hungary	2022	33	GCA_048287155.1
518-11/2022	Surface water	Hungary	Hungary	2022	33	GCA_048287235.1
573-11/2022	Surface water	Hungary	Hungary	2022	33	GCA_048287335.1
CLFF/3806/22 torn/5623/16	Rosa sp.	Poland	Poland	2022	33	GCA_048285755.1
PD 1255	Rosa sp.	Poland	Poland	2016	33	GCA_048285255.1
PD 1255	Anthurium andraeanum	France (Reunion)	Netherlands	1988	33	GCA_049726525.1
PD 3597	Solanum lycopersicum	Kenya	Netherlands	1999	33	GCA_048285775.1
PD 3634	Solanum tuberosum	Kenya	Netherlands	1999	33	GCA_049726275.1
PD 5415	Anthurium andraeanum	Ghana	Netherlands	2006	33	GCA_048285475.1
PD 7123	Rosa sp.	Netherlands	Netherlands	2015	33	GCA_048285495.1
GBBC 3080	Rosa sp.	Netherlands	Belgium	2015	33	GCA_040183095.1
Rpseud-SA1	Rosa sp.	Greece	Greece	2021	33	GCA_048285395.1
Rpseud-SP3	Rosa sp.	Greece	Greece	2021	33	GCA_048285375.1
PD 7413	Rosa sp.	Russia	Netherlands	2018	33	GCA_048284555.1
PD 7424	Rosa sp.	Russia	Netherlands	2018	33	GCA_048284535.1
PD 7901	Surface water	Netherlands	Netherlands	2020	33	GCA_048285265.1
PD 7902	Surface water	Netherlands	Netherlands	2020	33	GCA_048284545.1
PD 8114	Surface water	Netherlands	Netherlands	2021	33	GCA_048286335.1
PD 8131	Surface water	Netherlands	Netherlands	2021	33	GCA_048285285.1
PD 8157	Solanum dulcamara	Netherlands	Netherlands	2021	33	GCA_048284485.1
PD 8184	Solanum dulcamara	Netherlands	Netherlands	2021	33	GCA_048285185.1
PD 8473	Surface water	Netherlands	Netherlands	2023	33	GCA_048285845.1
Rs-09-161	Solanum melongena	India	India	2009	33	GCA_000671335.1
PD 1256	Solanum melongena	France (Reunion)	Netherlands	1988	31	GCA_049726545.1
AGQB#27	Zingiber officinale	Peru	Germany	2021	30	GCA_048285555.1
AGQB#49*	Zingiber officinale	Peru	Germany	2022	30	GCA_048285075.1
AGQB#54*	Zingiber officinale	Thailand	Germany	2022	30	GCA_048286315.1
AGQB#82*	Curcuma longa	Bangladesh	Germany	2022	30	GCA_048286375.1
AGQB#99*	Curcuma longa	Thailand	Germany	2023	30	GCA_048286435.1
AGQB#103	Zingiber officinale	China	Germany	2023	30	GCA_048287275.1
NIB Z 3683	Zingiber officinale	Peru	Slovenia	2023	30	GCA_048285795.1
NIB Z 3685	Zingiber officinale	Peru	Slovenia	2023	30	GCA_048284835.1
RC5-2 CH	Zingiber officinale	Peru	Switzerland	2023	30	GCA_048285485.1

**Table 2** (continued)

Isolate	Isolated from	Country of origin	Country of isolation	Year	Best matching sequevar	Assembly number
PD 2272	<i>Curcuma longa</i>	Netherlands (import) **	Netherlands	1993	30	GCA_048285365.1
PD 3196	<i>Curcuma longa</i>	Netherlands (import) **	Netherlands	1997	30	GCA_048285385.1
PD 3570	<i>Curcuma longa</i>	Thailand	Netherlands	1999	30	GCA_048286345.1
PD 3822	<i>Curcuma alismatifolia</i>	South Africa	South Africa	2000	30	GCA_048284565.1
PD 4138	<i>Curcuma longa</i>	Netherlands (import) **	Netherlands	2001	30	GCA_048285195.1
PD 5537	<i>Curcuma</i> sp.	Israel	Netherlands	2008	30	GCA_048286355.1
PD 5777	<i>Curcuma</i> sp.	Thailand	Netherlands	2009	30	GCA_048285505.1
PD 7098	<i>Curcuma</i> sp.	Netherlands (import) **	Netherlands	2015	30	GCA_048285235.1
PD 8575	<i>Curcuma</i> sp.	Netherlands (import) **	Netherlands	2014	30	GCA_048285155.1
PD 8621	<i>Zingiber officinale</i>	Peru	Netherlands	2024	30	GCA_048285815.1
CREA-DC 2104 (I2116)	<i>Solanum lycopersicum</i>	Italy	Italy	2022	18	GCA_048285735.1
CREA-DC 2105 (I2120)	<i>Solanum lycopersicum</i>	Italy	Italy	2022	18	GCA_048285175.1
GMI1000	<i>Solanum lycopersicum</i>	French Guiana	French Guiana	1968	18	GCA_000009125.1
AGQB#45	<i>Curcuma longa</i>	Costa Rica	Germany	2021	17	GCA_048285135.1
AGQB#48*	<i>Curcuma longa</i>	India	Germany	2022	17	GCA_048284515.1
AGQB#119*	<i>Curcuma longa</i>	Vietnam	Germany	2023	17	GCA_048285085.1
PD 3205	<i>Anthurium andraeanum</i>	Philippines	Netherlands	1997	17	GCA_049726405.1
UW640	<i>Zingiber officinale</i>	Australia	Australia	unknown	16	GCA_048285355.1
PD1419	<i>Solanum tuberosum</i>	Japan	Japan	1989	15	GCA_049726445.1
AGQB#125*	<i>Zingiber officinale</i>	China	Germany	2024	14	GCA_048285455.1
PD 3278	<i>Morus</i> sp.	China	China	1998	12	GCA_049726425.1

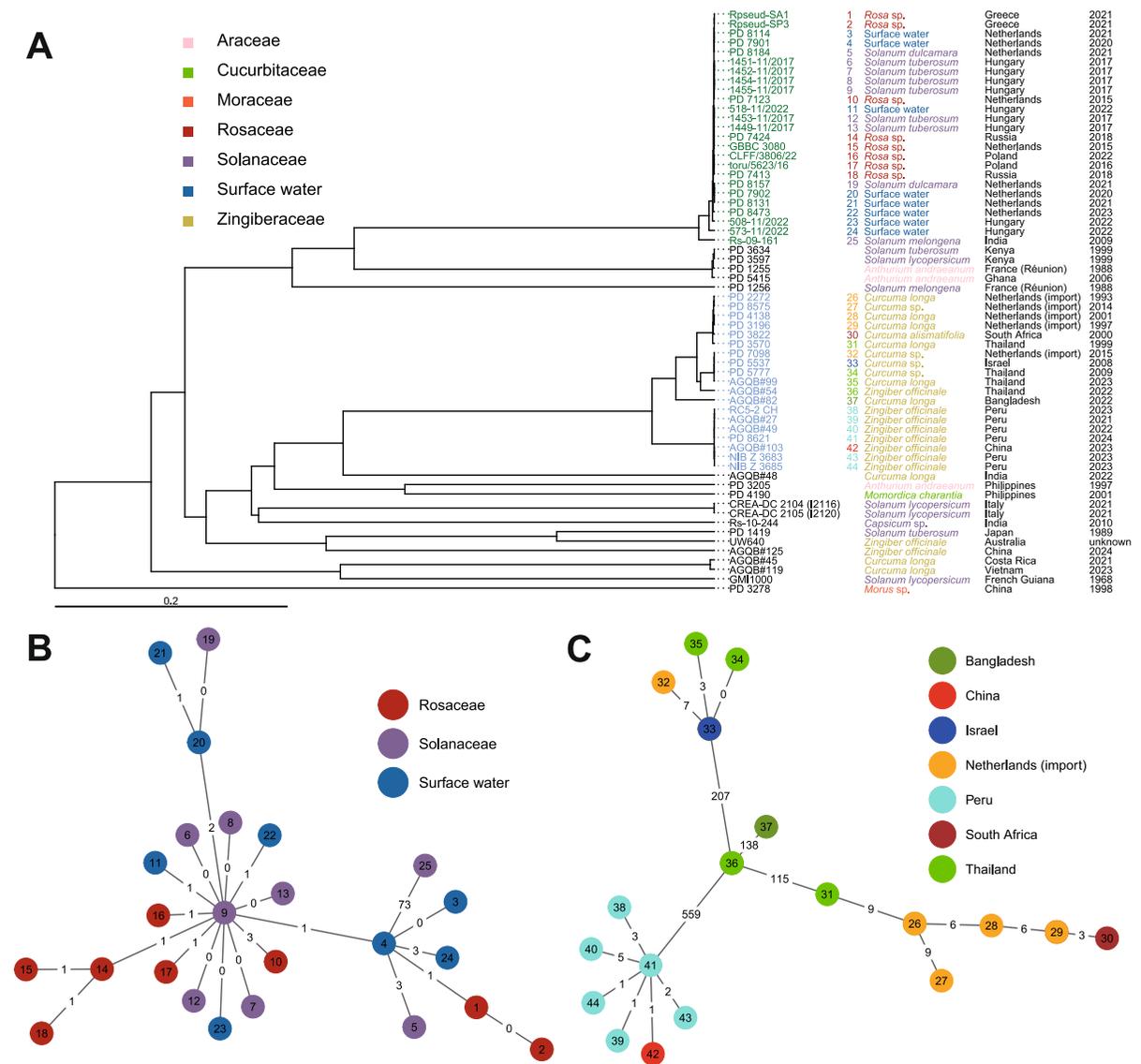
\* = interception at border control point

\*\* = interception at border control point, country of origin unknown

group 1 (isolates 26–31), group 2 (32–35) and group 3 (38–44), and singleton isolates AGQB#54 (36) and AGQB#82 (37) (corresponding isolate names are depicted in Fig. 2A). The number of loci with at least one SNP difference among the three distinct clonal lineages in the "major ginger clade" ranged from 115 to 559 (Fig. 2C), which supports the idea that this clade has a higher level of genomic diversity.

To evaluate the biological relevance of the genetic diversity observed among the *R. pseudosolanacearum* phy I isolates associated with recent European findings, pathogenicity assays were conducted on *S.*

*lycopersicum* (tomato) cv. 'Moneymaker'. Two inoculation methods were employed: 1. injecting a bacterial suspension ( $10^6$ – $10^8$  CFU/mL in 10 mM PB) at the base of a tomato stem just below the first true leaves with a sterile syringe with a needle and incubation of the young tomato plants at 27–28/20 °C (day/night) under a 16/8 h light/dark cycle and 70–80% relative humidity (%RH), or 2. direct needle/pin-prick inoculation from a bacterial colony and incubation of the young tomato plants at a 28/25 °C day/night temperature under similar light/dark and %RH conditions. Symptom development was monitored



**Fig. 2** Phylogenetic and core genome multilocus sequence typing (cgMLST) analysis of *R. pseudosolanacearum* phy I isolates. (A) A phylogenetic tree was constructed using hierarchical clustering (UPGMA method) of ANI values. Isolates belong to the “rose” and “major ginger” clade are numerically labeled (1–44), corresponding to labels in panels B and C. Isolate designation for isolates belonging to the “rose clade” is given in green, while for “major ginger clade” isolates is given in blue. The matrix, country of origin, and year of isolation for

each isolate are indicated, with matrix colours corresponding to the legend on the left. Minimum spanning trees illustrate the cgMLST-based relationships among “rose clade” (panel B) and “major ginger clade” (panel C) isolates, respectively. Each circle represents an isolate, numbered as in panel A. The circle colour indicates the matrix from which the isolate was obtained (B) or the country of origin (C). Numbers on the connecting lines (edges) represent the number of allelic differences between isolates

and recorded qualitatively for up to 14 days post-inoculation (dpi). All *R. pseudosolanacearum* phy I isolates included in the pathogenicity assays were highly virulent on tomato regardless of their original host (Table 3; Fig. 3). Typical *Ralstonia* symptoms,

including rapid wilting and necrosis, manifested in 50–100% of the inoculated plants. Initial signs of leaf wilting typically appeared within 1–7 dpi, except for AGQB#103, which had a delayed wilt onset of 10 days (Table 3). The pathogen’s identity was

**Table 3** Results from the pathogenicity tests upon inoculation of *R. pseudosolanacearum* phy I in young tomato plants (cv. Money-maker)

<i>R. pseudosolanacearum</i> phy I isolate	Plant/Matrix	# inoculated plants	# inoculated plants with symptoms	% plants with symptoms	# dpi that first symptoms observed
PD 8575	Curcuma sp	10	5	50	4
PD 7192	water (from greenhouse rose cultivation)	10	10	100	4
PD 7123	<i>Rosa</i> sp	10	10	100	3
PD 8565	<i>Rosa</i> sp	10	10	100	5
PD 7413	<i>Rosa</i> sp	10	10	100	5
PD 7424	<i>Rosa</i> sp	10	10	100	5
PD 7901	surface water	10	10	100	6
PD 7902	surface water	10	10	100	6
PD 8114	surface water	10	10	100	3
PD 8131	surface water	10	10	100	7
PD 8157	<i>S. dulcama</i>	10	9	90	4
PD 8184	<i>S. dulcama</i>	10	10	100	4
PD 8621	<i>Zingiber officinale</i>	10	10	100	7
GBBC 3080	<i>Rosa</i> sp	10	10	100	3
AGQB#27	<i>Zingiber officinale</i>	10	7	70	4
AGQB#48	<i>Curcuma longa</i>	10	10	100	4
AGQB#103	<i>Zingiber officinale</i>	4	4	100	10
NIB Z 3683-D1560/23–8	<i>Zingiber officinale</i>	5	5	100	1
NIB Z 3685	<i>Zingiber officinale</i>	5	5	100	1

**Fig. 3** Bacterial wilting and necrosis observed on young tomato plants (cv. Moneymaker) inoculated with *R. pseudosolanacearum* phy I at 7 dpi (A) Negative control (PBS

0,1 M). (B) Plant inoculated with isolate AGQB#27 (seq 30) from ginger. (C) Plant inoculated with isolate AGQB#48 (seq 17) from curcuma

confirmed by its successful re-isolation on the semi-selective mSMSA medium, followed by its identification using TaqMan® real-time PCR (Vreeburg et al., 2016), IF testing, or MALDI-TOF MS (van de Bilt et al., 2018), thus fulfilling Koch's postulates.

Bacterial wilt caused by *R. pseudosolanacearum* phy I is one of the most destructive bacterial plant diseases in tropical and subtropical climates (Subedi et al., 2020). It is particularly challenging to manage due to its high genetic diversity, its ability to colonize

hosts systemically, and its ability to persist in a wide range of agricultural and environmental niches (Vail-leau & Genin, 2023). Between 2015 and 2024, the number of *R. pseudosolanacearum* phy I findings increased across Europe. These were linked to either infected plant material at import (interceptions), to plants cultivated in specific European regions, often after importation of infected plant material, or to reservoirs such as natural aquatic environments (Tables 1 and 2).

Our comparative genomic analysis of 62 *R. pseudosolanacearum* phy I isolates, including those recently found in various European countries, revealed several distinct genetic clades. Three clades are particularly noteworthy: the "rose clade", the "major ginger clade" and the "tomato clade". The "major ginger clade" showed the highest level of genetic diversity among the isolates, comprising three clonal lineages and two divergent isolates (groups 1 to 3, and isolates AGQB#54 and AGQB#82, Fig. 2C). No consistent association could be established between clonal lineages and the geographical origin of the isolates. Furthermore, the "major ginger clade" did not contain all recently detected *R. pseudosolanacearum* phy I isolates from ginger and curcuma, with four isolates falling outside this clade. In contrast, the "rose clade" exhibited minimal variation, with recent isolates appearing clonal (Fig. 2B). The closest relative to the "rose clade" identified in this analysis was Rs-09–161, a *R. pseudosolanacearum* phy I isolate from eggplant in India in 2009 (Ramesh et al., 2014).

Despite the growing availability of genomic data and a recent study on molecular epidemiology (Cellier et al., 2023a, 2023b), our understanding of the population dynamics of *R. pseudosolanacearum* phy I at the field level remains limited (Yahiaoui et al., 2017). However, the persistence of this pathogen in aquatic environments for multiple successive years suggests that its establishment in Europe is likely (Vogelaar et al., 2023). This risk is expected to increase with climate change, given that *R. pseudosolanacearum* phy I proliferates optimally at temperatures above 30°C, with an optimal metabolic capacity at 35°C (Wang et al., 2020). This aligns with IPCC predictions that temperature shifts will significantly affect plant pathogen dynamics (IPCC Secretariat, 2022).

The invasive potential of *R. pseudosolanacearum* phy I to new environments might be further enhanced by its wide host range and its broad virulence (He et al., 2021; Vaillau & Genin, 2023). Our findings demonstrate that, irrespective of the original host, European *R. pseudosolanacearum* phy I isolates consistently exhibit high virulence on tomato, including rapid wilting and necrosis in 50–100% of the inoculated plants. Although more natural and less aggressive inoculation methods might give different results these results are consistent with previous

reports of highly virulent *R. pseudosolanacearum* phy I isolates in other Solanaceous plants (Tjou-Tam-Sin et al., 2017b), including potato (Overeem et al., 2023). Similarly, rose plants (cv. 'Aqua' or cv. 'Papa Meilland') exhibited chlorosis and necrosis of leaves at 17 dpi (Holeva et al., 2024), and plant death at 28°C already after 18 dpi (cv. 'Armando') upon stem inoculation (Tjou-Tam-Sin et al., 2017b). In some of our pathogenicity experiments, European *R. pseudosolanacearum* phy I isolates were more virulent on tomato than *R. solanacearum* phy II isolates (data not shown). This is consistent with earlier findings that a higher proportion of *R. pseudosolanacearum* phy I isolates can overcome partial resistance in crops such as the scarlet eggplant (*Solanum aethiopicum*) (Silva et al., 2024).

In conclusion, the increasing prevalence of *R. pseudosolanacearum* phy I in European agricultural areas requires greater awareness and coordinated phytosanitary action. This pathogen can affect and persist in a wide range of host plants and environmental reservoirs (Lebeau et al., 2011; Lopes & Rossato, 2018; Santiago et al., 2017; Vaillau & Genin, 2023; Wicker et al., 2012). At the EU level, official phytosanitary measures should focus on preventing the entry of infected plant material, e.g. through strengthened surveillance and early detection. This is important since the introduced isolates show relatively large genetic variability and thus new introduction might come with new risks, e.g. different host range or climate adaptation. Import control should be primarily focused on propagative plant material, however, based on the German experiences, plant material designated for consumption that could be used as planting material might be considered as well. Furthermore, efforts should be made to prevent local spread, for instance through annual surveys of high-risk host plants and waterways. Although the pathogen is present in some parts of Europe it is not widespread. Once *R. pseudosolanacearum* phy I becomes widely established, eradication or containment would be extremely challenging, if not impossible.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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## References

- Álvarez, B., Biosca, E. G., & López, M. M. (2010). On the life of *Ralstonia solanacearum*, a destructive bacterial plant pathogen. In A. Méndez-Vilas (Ed.), *Current research, technology and education topics in applied microbiology and microbial biotechnology* (pp. 267–269). Formatex.
- Bergsma-Vlami, M., van de Bilt, J. L. J., Tjou-Tam-Sin, N. N. A., Westenberg, M., Meekes, E. T. M., Teunissen, H. A. S., & Van Vaerenbergh, J. (2018). Phylogenetic assignment of *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* phylotype I) isolated from *Rosa* spp. *Plant Disease*, 102(11), 2258–2267. <https://doi.org/10.1094/PDIS-09-17-1345-RE>
- Bocsanczy, A. M., Achenbach, U. C. M., Mangravita-Novo, A., Yuen, J. M. F., & Norman, D. J. (2012). Comparative effect of low temperature on virulence and twitching motility of *Ralstonia solanacearum* strains present in Florida. *Phytopathology*, 102(2), 185–194. <https://doi.org/10.1094/PHYTO-05-11-0145>
- Bocsanczy, A. M., Huguet-Tapia, J. C., & Norman, D. J. (2017). Comparative genomics of *Ralstonia solanacearum* identifies candidate genes associated with cool virulence. *Frontiers in Plant Science*, 8, Article 1565. <https://doi.org/10.3389/fpls.2017.01565>
- Briatte, F. (2024). ggnetwork: Geometries to plot networks with “ggplot2.” <https://github.com/briatte/ggnetwork>
- Cellier, G., Nordey, T., Cortada, L., Gauche, M., Rasoamana, H., Yahiaoui, N., Rébert, E., Prior, P., Chéron, J. J., Poussier, S., & Pruvost, O. (2023a). Molecular epidemiology of *Ralstonia pseudosolanacearum* phylotype I strains in the Southwest Indian Ocean region and their relatedness to African strains. *Phytopathology*, 113(3), 423–435. <https://doi.org/10.1094/PHYTO-09-22-0355-R>
- Cellier, G., Pecrix, Y., Gauche, M. M., & Cheron, J. J. (2023b). *Ralstonia solanacearum* species complex egl reference database. CIRAD Dataverse. <https://doi.org/10.18167/DVNI/CUWA5P>
- Csárdi, G., Nepusz, T., Müller, K., Horvát, S., Traag, V., Zanini, F., & Noom, D. (2024). igraph for R: R interface of the igraph library for graph theory and network analysis. *Zenodo*. <https://doi.org/10.5281/ZENODO.10681749>
- Dewberry, R. J., Sharma, P., Prom, J. L., Kinscherf, N. A., Lowe-Power, T., Mazloom, R., Zhang, X., Liu, H., Arif, M., Stulberg, M., Heath, L. S., Eversole, K., Beattie, G. A., Vinatzer, B. A., & Allen, C. (2024). Genotypic and phenotypic analyses show *Ralstonia solanacearum* cool virulence is a quantitative trait not restricted to “race 3 biovar 2.” *Phytopathology*, 114(12), 2468–2480. <https://doi.org/10.1094/PHYTO-06-24-0187-R>
- EFSA Panel on Plant Health (EFSA PLH Panel), Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jaques Miret, J. A., Justesen, A. F., MacLeod, A., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Reignault, P. L., Thulke, H., Van der Werf, W., Vicent Civera, A., Yuen, J., et al. (2019). Scientific opinion on the pest categorisation of the *Ralstonia solanacearum*

- species complex. *EFSA Journal*, 17(2), 5618. <https://doi.org/10.2903/j.efsa.2019.5618>
- Elphinstone, J. G. (2005). The current bacterial wilt situation: A global overview. In C. Allen, P. Prior, & A. C. Hayward (Eds.), *Bacterial wilt disease and the Ralstonia solanacearum species complex* (pp. 9–28). APS Press.
- Elphinstone, J. G., Hennessy, J., Wilson, J. K., & Stead, D. E. (1996). Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *EPPO Bulletin*, 26(3–4), 663–678. <https://doi.org/10.1111/j.1365-2338.1996.tb01511.x>
- EPPO. (2025). EPPO global database. <https://gd.eppo.int>. Accessed 12 November 2025
- European Commission. (2022). Commission Implementing Regulation (EU) 2022/1193 of 11 July 2022 establishing measures to eradicate and prevent the spread of *Ralstonia solanacearum* (Smith 1896) Yabuuchi et al. 1996 emend. Safni et al. 2014. Official Journal of the European Union. [http://data.europa.eu/eli/reg\\_impl/2022/1193/oj](http://data.europa.eu/eli/reg_impl/2022/1193/oj)
- European Commission. (2024). Commission Implementing Regulation (EU) 2024/591 of 20 February 2024 amending Implementing Regulation (EU) 2022/2389 concerning the establishment of frequency rates for identity checks and physical checks on consignments of plants, plant products and other objects entering the Union. *Official Journal of the European Union*. [http://data.europa.eu/eli/reg\\_impl/2024/591/oj](http://data.europa.eu/eli/reg_impl/2024/591/oj)
- Fegan, M., & Prior, P. (2005). How complex is the “*Ralstonia solanacearum* species complex.” In C. Allen, P. Prior, & A. C. Hayward (Eds.), *Bacterial wilt disease and the Ralstonia solanacearum species complex* (pp. 449–461). APS Press.
- Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist*, 187(4), 920–928. <https://doi.org/10.1111/j.1469-8137.2010.03397.x>
- Genin, S., & Denny, T. P. (2012). Pathogenomics of the *Ralstonia solanacearum* species complex. *Annual Review of Phytopathology*, 50(1), 67–89. <https://doi.org/10.1146/annurev-phyto-081211-173000>
- He, Y., Chen, Y., Zhang, Y., Qin, X., Wei, X., Zheng, D., Lin, W., Li, Q., & Yuan, G. (2021). Genetic diversity of *Ralstonia solanacearum* species complex strains obtained from Guangxi, China and their pathogenicity on plants in the Cucurbitaceae family and other botanical families. *Plant Pathology*, 70(6), 1445–1454. <https://doi.org/10.1111/ppa.13389>
- Holeva, M. C., Glynos, P. E., Reppa, C. I., & Karafra, C. D. (2024). First report of *Ralstonia pseudosolanacearum* causing bacterial wilt on *Rosa* sp. in Greece. *Plant Disease*, 108(4), 1095. <https://doi.org/10.1094/PDIS-11-23-2279-PDN>
- IPPC Secretariat. (2022). 2021 IPPC annual report - protecting the world’s plant resources from pests. Rome: FAO on behalf of the Secretariat of the International Plant Protection Convention. <https://doi.org/10.4060/cb9334en>
- Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, 37(5), 540–546. <https://doi.org/10.1038/s41587-019-0072-8>
- Lebeau, A., Daunay, M.-C., Frary, A., Palloix, A., Wang, J.-F., Dintinger, J., Chiroleu, F., Wicker, E., & Prior, P. (2011). Bacterial wilt resistance in tomato, pepper, and eggplant: Genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology*, 101(1), 154–165. <https://doi.org/10.1094/PHYTO-02-10-0048>
- Lelliott, R. A., & Stead, D. E. (1987). *Methods for the diagnosis of bacterial diseases of plants*. Blackwell Scientific Publications.
- Lopes, C. A., & Rossato, M. (2018). History and status of selected hosts of the *Ralstonia solanacearum* species complex causing bacterial wilt in Brazil. *Frontiers in Microbiology*, 9, Article 1228.
- Lowe-Power, T. M., Avalos, J., Bai, Y., Munoz, M. C., Chipman, K., Elmgreen, V. N., Prasad, N., Ramirez, B., Sandhar, A., Tom, C. E., & Williams, D. (2024). A meta-analysis of the known global distribution and host range of the *Ralstonia* species complex. *Microbiology*. <https://doi.org/10.1101/2020.07.13.189936>
- Overeem, R., Pel, C., Tjou-Tam-Sin, N., van de Bilt, J., Gorkink-Smits, P., Landman, M., Bocsanczy, A. M. B., Norman, D., & Bergsma-Vlami, M. (2023). Virulence of novel *Ralstonia pseudosolanacearum* (phyloptype I) strains from rose, blueberry, and mandevilla on seed potato. *Plant Disease*, 107(12), 3718–3726. <https://doi.org/10.1094/pdis-12-22-2931-sr>
- Paradis, E., & Schliep, K. (2019). Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35(3), 526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Pastrik, K.-H., Elphinstone, J. G., & Pukall, R. (2002). Sequence analysis and detection of *Ralstonia solanacearum* by multiplex PCR amplification of 16S–23S ribosomal intergenic spacer region with internal positive control. *European Journal of Plant Pathology*, 108(9), 831–842. <https://doi.org/10.1023/a:1021218201771>
- Paudel, S., Dobhal, S., Alvarez, A. M., & Arif, M. (2020). Taxonomy and phylogenetic research on *Ralstonia solanacearum* species complex: A complex pathogen with extraordinary economic consequences. *Pathogens*, 9(11), Article 886. <https://doi.org/10.3390/pathogens9110886>
- Prior, P., Ailloud, F., Dalsing, B. L., Remenant, B., Sanchez, B., & Allen, C. (2016). Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC Genomics*, 17(1), Article 90. <https://doi.org/10.1186/s12864-016-2413-z>
- Pritchard, L., Glover, R. H., Humphris, S., Elphinstone, J. G., & Toth, I. K. (2016). Genomics and taxonomy in diagnostics for food security: Soft-rotting enterobacterial plant pathogens. *Analytical Methods*, 8(1), 12–24. <https://doi.org/10.1039/C5AY02550H>
- Prijbelski, A., Antipov, D., Meleshko, D., Lapidus, A., & Korobeynikov, A. (2020). Using SPAdes de novo assembler. *Current Protocols in Bioinformatics*, 70(1), Article e102. <https://doi.org/10.1002/cpbi.102>
- Ramesh, R., Achari, G. A., & Gaitonde, S. (2014). Genetic diversity of *Ralstonia solanacearum* infecting solanaceous vegetables from India reveals the existence of unknown or newer sequenvars of phyloptype I strains. *European Journal*

- of *Plant Pathology*, 140(3), 543–562. <https://doi.org/10.1007/s10658-014-0487-5>
- Rivera-Zuluaga, K., Hiles, R., Barua, P., Caldwell, D., & Iyer-Pascuzzi, A. S. (2023). Getting to the root of *Ralstonia* invasion. *Seminars in Cell & Developmental Biology*, 148, 3–12. <https://doi.org/10.1016/j.semcdb.2022.12.002>
- Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., & Kappler, U. (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: Proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. nov., *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 64, 3087–3103. <https://doi.org/10.1099/ijs.0.066712-0>
- Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J. C., Cattolico, L., Chandler, M., Choisine, N., Claudel-Renard, C., Cunnac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., et al. (2002). Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, 415(6871), 497–502. <https://doi.org/10.1038/415497a>
- Santiago, T. R., Lopes, C. A., Caetano-Anollés, G., & Mizubuti, E. S. G. (2017). Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. *Plant Pathology*, 66(3), 383–392. <https://doi.org/10.1111/ppa.12586>
- Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3(3), 430–439. <https://doi.org/10.1038/s41559-018-0793-y>
- Sharma, P., Johnson, M. A., Mazloom, R., Allen, C., Heath, L. S., Lowe-Power, T. M., & Vinatzer, B. A. (2022). Meta-analysis of the *Ralstonia solanacearum* species complex (RSSC) based on comparative evolutionary genomics and reverse ecology. *Microbial Genomics*. <https://doi.org/10.1099/mgen.0.000791>
- Silva, P. H. R., Johnson-Silva, W., Albuquerque, G. M. R., Oliveira, V. L. B., Santos, L. V. S., Gonçalves, M. H. O., Gama, M. A. S., & Souza, E. B. (2024). Exploring the competitive potential of *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum*: Insights from a comparative adaptability study. *Plant Pathology*, 73(4), 898–914. <https://doi.org/10.1111/ppa.13848>
- Silva, M., Machado, M. P., Silva, D. N., Rossi, M., Moran-Gilad, J., Santos, S., Ramirez, M., & Carriço, J. A. (2018). chewBBACA: A complete suite for gene-by-gene schema creation and strain identification. *Microbial Genomics*, 4(3). <https://doi.org/10.1099/mgen.0.000166>
- Souvorov, A., Agarwala, R., & Lipman, D. J. (2018). SKESA: Strategic k-mer extension for scrupulous assemblies. *Genome Biology*, 19(1), Article 153. <https://doi.org/10.1186/s13059-018-1540-z>
- Subedi, N., Taylor, C. G., Paul, P. A., & Miller, S. A. (2020). Combining partial host resistance with bacterial biocontrol agents improves outcomes for tomatoes infected with *Ralstonia pseudosolanacearum*. *Crop Protection*, 135, Article 104776. <https://doi.org/10.1016/j.cropro.2019.03.024>
- Subedi, N., Cowell, T., Cope-Arguello, M., Paul, P., Cellier, G., Bkayrat, H., Bonagura, N., Cadatal, A., Chen, R., Enriquez, A., Parasar, R., Repetto, L., Rivas, A. H., Shahbaz, M., White, K., Lowe-Power, T. M., & Miller, S. A. (2024). Characterization of *Ralstonia pseudosolanacearum* diversity and screening tomato, pepper, and eggplant resistance to manage bacterial wilt in South Asia. *PhytoFrontiers™*, 4(3), 393–403. <https://doi.org/10.1094/PHYTOFR-10-23-0136-R>
- Tjou-Tam-Sin, N. N. A., van de Bilt, J. L. J., Westenberg, M., Bergsma-Vlami, M., Korpershoek, H. J., Vermunt, A. M. W., Meekes, E. T. M., Teunissen, H. A. S., & Van Vaerenbergh, J. (2017a). First report of bacterial wilt caused by *Ralstonia solanacearum* in ornamental *Rosa* sp. *Plant Disease*, 101(2), 378–378. <https://doi.org/10.1094/PLDIS-02-16-0250-PDN>
- Tjou-Tam-Sin, N. N. A., van de Bilt, J. L. J., Westenberg, M., Gorkink-Smits, P. P. M. A., Landman, N. M., & Bergsma-Vlami, M. (2017b). Assessing the pathogenic ability of *Ralstonia pseudosolanacearum* (*Ralstonia solanacearum* phylotype I) from ornamental *Rosa* spp. plants. *Frontiers in Plant Science*, 8, Article 1895. <https://doi.org/10.3389/fpls.2017.01895>
- Vailleau, F., & Genin, S. (2023). *Ralstonia solanacearum*: An arsenal of virulence strategies and prospects for resistance. *Annual Review of Phytopathology*, 61(1), 25–47. <https://doi.org/10.1146/annurev-phyto-021622-104551>
- van de Bilt, J. L. J., Wolsink, M. H. L., Gorkink-Smits, P. P. M. A., Landman, N. M., & Bergsma-Vlami, M. (2018). Application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry for rapid and accurate identification of *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum*. *European Journal of Plant Pathology*, 152(4), 921–931. <https://doi.org/10.1007/s10658-018-1517-5>
- Vogelaar, M. A. W., van de Bilt, J. L. J., Blom, N. I., Pel, M. J. C., van Doorn, B. J. A., Landman, N. M., Gorkink-Smits, P. P. M. A., Raaymakers, T. M., Vreeburg, R. A. M., & Bergsma-Vlami, M. (2023). Presence of *Ralstonia pseudosolanacearum* (phylotype I) in aquatic environments in the Netherlands. *Plant Disease*, 107(8), 2320–2324. <https://doi.org/10.1094/PLDIS-11-22-2628-SC>
- Vreeburg, R. A. M., Bergsma-Vlami, M., Bollema, R. M., de Haan, E. G., Kooman-Gersmann, M., Smits-Mastebroek, L., Tameling, W. I. L., Tjou-Tam-Sin, N. N. A., van de Vossen, B. T. L. H., & Janse, J. D. (2016). Performance of real-time PCR and immunofluorescence for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum* in potato tubers in routine testing. *EPPO Bulletin*, 46(1), 112–121. <https://doi.org/10.1111/epp.12278>
- Vreeburg, R. A. M., Zendman, A. J. W., Pol, A., Verheij, E., Nas, M., & Kooman-Gersmann, M. (2018). Validation of four real-time TaqMan PCRs for the detection of *Ralstonia solanacearum* and/or *Ralstonia pseudosolanacearum* and/or *Clavibacter michiganensis* subsp. *sepedonicus* in potato tubers using a statistical regression approach.

- EPP0 Bulletin*, 48(1), 86–96. <https://doi.org/10.1111/epp.12444>
- Wang, H.-C., Guo, H., Cai, L., Cai, L.-T., Guo, Y.-S., & Ding, W. (2020). Effect of temperature on phenotype characterization of *Ralstonia solanacearum* from tobacco. *Canadian Journal of Plant Pathology*, 42(2), 164–181. <https://doi.org/10.1080/07060661.2019.1654547>
- Weller, S. A., Elphinstone, J. G., Smith, N. C., Boonham, N., & Stead, D. E. (2000). Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Applied and Environmental Microbiology*, 66(7), 2853–2858. <https://doi.org/10.1128/AEM.66.7.2853-2858.2000>
- Wick, R. R., Howden, B. P., & Stinear, T. P. (2025). Auto-cycler: Long-read consensus assembly for bacterial genomes. *Bioinformatics*, 41(9), Article btaf474. <https://doi.org/10.1093/bioinformatics/btaf474>
- Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., & Prior, P. (2007). *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. *Applied and Environmental Microbiology*, 73(21), 6790–6801. <https://doi.org/10.1128/AEM.00841-07>
- Wicker, E., Lefeuvre, P., de Cambiaire, J.-C., Lemaire, C., Poussier, S., & Prior, P. (2012). Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. *The ISME Journal*, 6(5), 961–974. <https://doi.org/10.1038/ismej.2011.160>
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemond, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., et al. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, 4(43), Article 1686. <https://doi.org/10.21105/joss.01686>
- Yahiaoui, N., Chéron, J.-J., Ravelomanantsoa, S., Hamza, A. A., Petrousse, B., Jeetah, R., Jaufeerally-Fakim, Y., Félicité, J., Fillâtre, J., Hostachy, B., Guérin, F., Cellier, G., Prior, P., & Poussier, S. (2017). Genetic diversity of the *Ralstonia solanacearum* species complex in the South-west Indian Ocean islands. *Frontiers in Plant Science*, 8, Article 2139. <https://doi.org/10.3389/fpls.2017.02139>
- Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T. (2017). GGTREE: An R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, 8(1), 28–36. <https://doi.org/10.1111/2041-210X.12628>

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