Deciphering Phenotypic Diversity of *Ralstonia solanacearum* Strains Pathogenic to Potato

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Accepted for publication 20 July 2010.

ABSTRACT

Cellier, G., and Prior, P. 2010. Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. *Phytopathology* 100:1250-1261.

Based on the phylotype classification, we questioned how genetically and phenotypically diverse strains of *Ralstonia solanacearum* pathogenic to potato may be. We studied 129 European and Mediterranean strains along with 57 reference strains known to cover genetic diversity in this species. Phylogeny analysis was done on endoglucanase gene sequences. Pathogenicity to potato, tomato, and eggplant was established at 24 to 30°C and 15 to 24°C, whereas tests on banana were conducted at 24 to 30°C. The ability to cause wilt on species of *Solanaceae* was shared by strains in all four phylotypes. Brown rot phylotypes IIB-1 and IIB-2 and

phylotype IIB-27 established latent infections in banana, and Moko disease-causing phylotypes IIA-6, IIB-3, and IIB-4 were virulent to susceptible potato and tomato, addressing the question of host adaptation mechanisms, which may have undergone a similar bottleneck evolution. Cold-tolerance ability is only shared on species of *Solanaceae* among brown rot phylotype IIB-1, which gathered the majority of European and Mediterranean strains. We surveyed strain LNPV24.25 as the first report of an emerging phylotype IIB-4NPB strain in France. These findings showed that pathogenicity traits of genetically identified strains still need to be understood, especially in the perspective of post-genomics comparative analysis, to understand bacterial speciation in the *R. solanacearum* species complex.

Ralstonia solanacearum is a soilborne plant-pathogenic β -proteobacterium with a wide host range and geographic distribution (17). Plants in at least 54 botanical families are affected in tropical and subtropical midland regions of the world (17–19). Strains within the species are heterogeneous and form part of a species complex (10). Bacterial wilt (BW) caused by *R. solanacearum* (Smith) (42) and recent introduction of brown rot strains in 14 of 27 Europe member states where outbreaks of brown rot have been reported, including The Netherlands, England, Belgium, and France (9,14,20), pose a serious threat to most cash and subsistence crops in the family *Solanaceae*, especially potato, tomato, sweet pepper, and tobacco. Major economic losses (9) have led to the status of the pathogen as an A2-listed EPPO quarantine pest (<http://www.eppo.org/QUARANTINE/listA2.htm>) in Europe and the bacterium being considered as a bioterrorism select agent by relevant authorities in the United States (22).

The phylogeny-based classification of the *R. solanacearum* species complex established on the partial endoglucanase (*egl*) gene sequence was reported as a good indicator of relationships between strains (10–12) and it was shown to be congruent with organismal phylogeny based on pangenomic DNA microarray hybridization (16). Brown rot strains are clustered into phylotype IIB sequevar 1 (IIB-1) and phylotype IIB sequevar 2 (IIB-2), historically known as race 3 biovar 2 (R3bv2), and were first described as potato and tomato pathogenic but weakly virulent on other *Solanaceae* spp. (4). This group also presented a high clonal trend, especially for members which were isolated under tem-

perate climate (17,20,36,38), showed different aggressiveness on *Solanaceae* spp. (37), and were able to trigger wilt even at cold temperatures (25).

Among the wide phylogenetic classification of *R. solanacearum*, major trends of pathogenicity were described and little is known about the exact virulence and aggressiveness of these strains. *R. solanacearum* strains which cluster into phylotype I encompassed a majority of lowland (tropical) strains with a wide host range. Within phylotype II, highland and cold-tolerant potato brown rot strains (2) belong to phylotype IIB-1 and IIB-2 (10), while many other lowland strains (formerly R1bv2T) are also able to cause wilt on *Solanaceae* spp., including potato (17). Phylotype IIB encompasses two distinctive clusters of banana Moko disease-causing strains (formerly R2bv1): sequevar 3 (IIB-3) and sequevar 4 (IIB-4). Recently, a new variant of *R. solanacearum*, that is pathogenic to the *Solanaceae* family but not pathogenic to banana (IIB-4NPB), has been recognized and also clusters into phylotype IIB-4 (11,40). Two additional clusters—namely, sequevar 6 and sequevar 24—containing Moko disease-causing strains have been assigned to phylotype IIA. Phylotype IIA contains strains that generally have a broad host range and are primarily isolated from tropical regions (formerly R1bv1). African strains in the relatively newly described phylotype III have been reported to share some pathogenicity traits with brown rot strains, including pathogenicity to potato in the highland of West Cameroon (24). Although phylogenetically indistinguishable, strains clustered into the Indonesian phylotype IV-10 are represented by two distinct species adapted to different hosts: *R. solanacearum* strains pathogenic to both potato and tomato and the blood disease bacterium (BDB) that causes wilt only on banana (11). Closely related to phylotype IV-10, phylotype IV-9 strains are causal agents of the Sumatra disease of cloves, a bacterium transmitted by the tube-building cercopoids *Hindola fulva* in Sumatra and *H. striata* in Java (8).

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*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains a supplemental table.

doi:10.1094/PHYTO-02-10-0059

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Many plant pathogen genomes are now available and many more are being sequenced, including members of the *R. solanacearum* species complex (https://www.genoscope.cns.fr/agc/microscope/about/collabprojects.php?P_id=67). It is generally expected that mining genomic data will lead to major discoveries about the subtle interactions that occur between plants and pathogens, as recently reviewed by Alfano (1). Nevertheless, the *R. solanacearum* species complex encompasses an unusually large number of phenotypically and genetically diverse strains, and it has become obvious that any post-genomic initiative to decipher host specialization should primarily aim at unraveling diversity in phenotypes, with special attention to pathogenicity of strains. Using the phylotyping scheme and related diagnostic tools as a framework, we hypothesized that the genetic and pathogenic diversity of strains originating from Europe and the Mediterranean is much greater than expected. We focused on cold-

tolerant potato brown rot strains of phylotype IIB-1, a major lineage of strains that is phylogenetically closely related to lineages containing banana Moko disease-causing strains. Within phylotype II, which encompasses the widest genetic diversity within the *R. solanacearum* species complex, both the brown rot and Moko disease-causing lineages are reported to be epidemiologically adapted to a particular host. That said, in this post-genomic data-mining era, the purpose of this study was to revisit and identify virulence patterns of these phenotypically divergent but genotypically similar *R. solanacearum* species complex strains.

MATERIALS AND METHODS

Bacterial strains. A set of 210 *R. solanacearum* strains was obtained and 42 strains were selected (Table 1) to be part of the

TABLE 1. Strains of *Ralstonia solanacearum* used in the phylogenetic study based on sequence variations of partial endoglucanase (*egl*) gene

Strain ^a	RUN ^b	Origin, host	Phylotype		GenBank ^c
			PCR ^c	<i>egl</i> ^d	
GMI8044	585	Grenada, banana	IIA-6	IIA-6	GU295013
RUN394	394	Grenada, banana Bluggoe	IIA-6	IIA-6	GU295048
CFBP4822	646	Finland, tomato	II	IIA-7	GU294993
JS927	909	Porto Rico, tomato	II	IIA-7	GU295027
JS967	911	Kenya, potato	II	IIA-7	GU295028
CFBP4613	896	Brazil, potato	II	IIA-28	GU294982
PD1100	622	Egypt, potato	II	IIA-35	GU295043
CFBP3105	685	Peru, potato	II	IIA-39	GU294940
CFBP3858	689	Netherlands, potato	II	IIB-1	AF295259
CFBP3870	691	South Africa, potato	II	IIB-1	GU294949
CFBP4787	633	Portugal, potato	II	IIB-1	GU294986
CMR34	147	Cameroon, tomato	II	IIB-1	EF439750
JT516	160	Reunion, potato	II	IIB-1	AF295258
LNPV28.23	654	Reunion, potato	II	IIB-1	GU294927
PSS525	256	Taiwan, potato	II	IIB-1	EU407303
CFBP1410	461	Colombia, banana plantain	II	IIB-2	GU294936
CFBP3879	628	Colombia, potato	II	IIB-2	GU294954
CFBP1416	459	Costa Rica, banana plantain	II	IIB-3	GU294937
CIP417	264	Philippines, banana	IIB-3	IIB-3	GU295004
CIP418	265	Indonesia, peanut	IIB-3	IIB-3	GU295005
UW28	1366	Cyprus, potato	IIB-3	IIB-3	GU295054
UW9	914	Costa Rica, <i>Heliconia</i> sp.	IIB-3	IIB-3	AF295257
CFBP1184	908	Honduras, <i>Musa</i> sp.	IIB-4	IIB-4	GU294935
LNPV31.10	662	French Guiana, <i>Musa</i> sp.	IIB-4	IIB-4	GU294928
LNPV32.36	665	French Guiana, <i>Musa</i> sp.	IIB-4	IIB-4	GU294931
LNPV32.37	666	French Guiana, banana plantain	IIB-4	IIB-4	GU294932
LNPV32.40	668	French Guiana, <i>Musa</i> sp.	IIB-4	IIB-4	GU294933
UW160	588	Peru, banana plantain	IIB-4	IIB-4	GU295051
UW162	453	Peru, banana plantain	IIB-4	IIB-4	AF295256
UW170	262	Colombia, <i>Heliconia</i> sp.	IIB-4	IIB-4	DQ011550
IBSBF2001	981	Brazil, tomato	II	IIB-25	GU295017
UQRS607	1340	Iran, potato	II	IIB-25	GU295050
CFBP6778	289	Martinique, tomato	IIB-4NPB	IIB-4	EF371851
CFBP6797	277	Martinique, American nightshade	IIB-4NPB	IIB-4	EF371855
IBSBF1503	302	Brazil, cucumber	IIB-4NPB	IIB-4	EF371868
JY200	280	Martinique, anthurium	IIB-4NPB	IIB-4	EF371857
LNPV24.25	651	France, tomato	IIB-4NPB	IIB-4	GU295039
RUN432	432	French Guiana, water (irrigation)	IIB-4NPB	IIB-4	GU294926
CMR20	137	Cameroon, tomato	III	III-29	GU295006
MAFF301552	70	Japan, tomato	IV	IV-8	GU295042
CFBP6727	702	Indonesia, potato	IV	IV-10	GU294995
CFBP6728	703	Indonesia, potato	IV	IV-10	GU294996
Reference strains					
R288	90	China, mulberry	I	I-12	GQ907153
CFBP7058	215	Cameroon, huckleberry	I	I-13	EF439740
PSS81	258	Taiwan, tomato	I	I-14	FJ561066
PSS4	157	Taiwan, tomato	I	I-15	EU407264

(continued on next page)

^a Strains obtained from French and international collections.

^b Identification number of strains in the collection maintained at CIRAD Reunion Island.

^c Phylotypes assignment as determined by multiplex polymerase chain reaction (PCR).

^d Phylotypes and sequevars assignment as determined by variation analysis of the partial *egl* gene sequences.

^e GenBank accession number.

phylogenetic tree from different bacterial collections maintained at Centre de coopération Internationale en Recherche Agronomique pour le Développement (Saint Pierre, Reunion Island; Le Lamentin, Martinique, French West Indies), Laboratoire National de la Protection des Végétaux (Angers, France), Institut National de la Recherche Agronomique (Rennes, France), University of Queensland (Brisbane, Australia), and Collection Française de Bactéries Phytopathogènes (Angers, France). Strains used in this study were isolated from BW field outbreaks or following interception from quarantined material by national plant health inspection services in Europe and the Mediterranean (EuMr). Collected strains were purified (i) onto modified Granada and Sequeira semiselective solid medium (13,27) and (ii) on Kelman's triphenyltetrazolium chloride (TZC-Kelman's) solid medium complemented with 0.5 g of yeast extract (21). Cultures were streaked on plates and incubated for 48 h at 28°C, and a single characteristic *R. solanacearum* colony for each strain was

harvested and stored at -80°C on beads (Pro-Lab Diagnostics, Microbank, Toronto).

Multiplex polymerase chain reactions for strains identification. The multiplex polymerase chain reaction (Pmx-PCR), developed by Fegan and Prior (10), was used to confirm that strains were *R. solanacearum* and to identify the phylotype to which they belonged. Strains from phylotype II originating from EuMr were further characterized by using a second specific multiplex PCR for Moko disease-causing strains (Mmx-PCR) in the *Musa* phylotypes IIA sequevar 6 (IIA-6), IIB-3, IIB-4, or IIB-4NPB (29,40).

Sequencing of partial *egl* gene and phylogeny. Sequences of *egl* ($n = 186$) (Table 1) were either retrieved from GenBank ($n = 57$) or determined in this study ($n = 129$) from a selection of strains isolated or intercepted in EuMr with a special care to strains isolated from potato. PCR amplicons of 750 bp were amplified following previously published methods (10,12,24) and

TABLE 1. (continued from preceding page)

Strain ^a	RUN ^b	Origin, host	Phylotype		GenBank ^c
			PCR ^c	<i>egl</i> ^d	
ACH92	44	Australia, Ginger	I	I-16	AF295254
P11	337	China, peanut	I	I-17	FJ561068
GMI1000	54	French Guiana, tomato	I	I-18	AF295251
JT519	471	Reunion, <i>Pelargonium</i> sp.	I	I-31	GU295032
PSS219	257	Taiwan, tomato	I	I-34	FJ561167
O3	339	China, Olive tree	I	I-44	FJ561069
CIP365	47	Philippines, potato	I	I-45	GQ907151
MAD17	320	Madagascar, Pepper	I	I-46	GU295040
GMI8254	597	Indonesia, tomato	I	I-47	GU295014
M2	343	China, mulberry	I	I-48	FJ561067
UW181	454	Venezuela, banana plantain	IIA-6	IIA-6	GU295053
ICMP7963	55	Kenya, potato	II	IIA-7	AF295263
K60	65	USA, tomato	II	IIA-7	AF295262
IBSBF1900	301	Brazil, <i>Musa</i> sp.	II	IIA-24	EF371839
CIP301	45	Peru, potato	II	IIA-35	GU295003
IBSBF1546	298	Brazil, California fuchsia	II	IIA-35	EF371877
CFBP2957	27	Martinique, tomato	II	IIA-36	AF295265
CFBP6779	288	Martinique, <i>Canna indica</i>	II	IIA-38	EF371872
CFBP2958	28	Guadeloupe, tomato	II	IIA-39	AF295266
CFBP2968	58	Reunion, potato	II	IIA-39	EF371806
UW469	109	Brazil, potato	II	IIA-40	DQ657612
CFBP7032	150	Cameroon, tomato	II	IIA-41	EF439726
T1-UY	448	Uruguay, tomato	II	IIA-50	GU295049
CFBP7054	203	Cameroon, tomato	II	IIA-52	EF439725
IPO1609	1	Netherlands, potato	II	IIB-1	EF371814
UW551	449	Kenya, <i>Pelargonium</i> sp.	II	IIB-1	DQ657596
CFBP4611	894	Colombia, potato	II	IIB-2	GU294981
Molk2	74	Philippines, <i>Musa</i> sp.	IIB-3	IIB-3	EF371841
CFBP6783	17	Martinique, <i>Heliconia</i> sp.	IIB-4NPB	IIB-4	EF371852
UW163	586	Peru, banana plantain	IIB-4	IIB-4	GU295052
CIP10	40	Peru, potato	II	IIB-25	AF295260
CIP240	482	Brazil, potato	II	IIB-26	EF647739
IBSBF1712	299	Brazil, <i>Pelargonium</i> sp.	II	IIB-27	EF371833
NCPBP3987	79	Brazil, potato	II	IIB-28	AF295261
CFBP7014	297	Trinidad, anthurium	II	IIB-51	AF371831
JT525	60	Reunion, <i>Pelargonium</i> sp.	III	III-19	AF295272
MAD-29	332	Madagascar, potato	III	III-19	GU295041
CMR33	146	Cameroon, tomato	III	III-20	GU295007
J25	56	Kenya, potato	III	III-20	AF295279
NCPBP1018	479	Angola, potato	III	III-21	AF295271
NCPBP332	1298	Zimbabwe, potato	III	III-22	AF295276
CFBP3059	39	Burkina Faso, eggplant	III	III-23	AF295270
CMR15	133	Cameroon, tomato	III	III-29	EF439743
CMR32	145	Cameroon, huckleberry	III	III-29	EF439749
DGBBC1227	364	Guinea, potato	III	III-42	GU295011
DGBBC1125	369	Guinea, potato	III	III-43	GU295008
DGBBC1138	362	Guinea, potato	III	III-43	GU295009
CMR66	166	Cameroon, huckleberry	III	III-49	EF439729
MAFF301558	71	Japan, potato	IV	IV-8	DQ011558
R28	89	Indonesia, clove	IV	IV-9	DQ011552
PSI7	83	Indonesia, tomato	IV	IV-10	EF371804
R229	62	Indonesia, banana	IV	IV-10	GU295045
ACH732	14	Australia, tomato	IV	IV-11	GQ907150

sequenced by MACROGEN Korea (single-pass double-stranded analysis) using the Endo-F and the Endo-R primers. Sequences were manually edited, checked, and trimmed at the consensus start sequence 5'-ACGGCGAT-3' or 5'-ACGGCGGC-3' and the consensus end sequence 5'-CAGTGG-3'. Alignment was conducted under the ARB Package (23) on a Linux system and phylogenetic trees generated using the neighbor-joining (NJ) algorithm corrected using the Jukes-Cantor model. Tree topology was tested using bootstrap analysis of 2,000 resamplings of the data.

Pathogenicity testing. A subset of *R. solanacearum* strains ($n = 167$) for which *egl* gene sequences had previously been determined was tested for pathogenicity on different host plants. Cells were grown from a single -80°C freeze-stored bead by placing the bead in 4.5 ml of Luria-Bertani broth for 24 h at 28°C with 250 rpm agitation for aeration; A 50- μl volume was streaked on TZC-Kelman's medium plate. An isolated colony was selected to be restreaked and cultured for 24 to 36 h at 28°C on the same medium. A bacterial suspension in 10 mM Tris, pH 7.1 (7 to 9; Sigma-Aldrich, St. Louis) was made from the restreaked culture and adjusted to 10^8 CFU/ml as determined by measuring the optical density at 0.1 at 650 nm. Plants were inoculated at the three-leaf development stage by pouring 5 ml of suspension on lateral roots that had been severed along one side using a scalpel. Negative control plants consisted of pouring 10 mM Tris (pH 7.1) onto severed roots. Pathogenicity was assessed on 10 plants per strain–host–experimental combination for tomato and eggplant accessions, whereas pathogenicity to banana was tested on 5 plants/strain. Infected plants were placed in environmental growth chambers called Rotoplan (Strader, Pellouailles les Vignes, France). Rotoplans were agreed for different norms of security levels (NS) containment: nonexotic strains were incubated in growth chambers termed NS2 and quarantine exotic organisms in chambers termed NS3. Strains ($n = 87$) with a phylogenetic position previously recognized to be present on Reunion were considered to be without environmental risk and, therefore, were incubated under the standard security level NS2-Rotoplan. Strains exotic to Reunion ($n = 80$) were incubated in a high security quarantine level NS3-Rotoplan. Each strain–plant combination was separated from the other combinations using a watertight plastic bag to avoid cross-contaminations. Development of disease was visually assessed at 3-day intervals for wilting during 30 days. At the end of the assay, surviving asymptomatic plants were sampled by cutting a 2-cm stem segment at collar level, and analyzed for latent infection by *R. solanacearum* by isolating the strain on a TZC-Kelman's medium plate. Special care was given to the sampling of infected banana for re culturing *R. solanacearum* targeting the corm leaf (CL) junction. A 2-cm segment of the CL section was sampled from all asymptomatic banana plants 3 weeks postinoculation; the first circular leaf was removed to avoid external contamination. Samples were crushed in 5 ml of 10 mM Tris, pH 7.1, and left for 1 h. A 50- μl aliquot of each extract was plated onto Sequeira's and TZC-Kelman's medium and incubated at 28°C for 72 h. A strain was said to be virulent to a particular plant species when showing the capacity to produce wilt symptoms and death on at least one single plant. Thus, pathogenicity of a strain was described by using two criteria: infections that resulted in plant death (wilt) and those that turned latent in a healthy plant carrier.

Pathogenicity assay of *R. solanacearum*. Tests were performed on potato cv. Bintje (Munterschen $\times$ Franschen) provided by the Fédération Nationale des Producteurs de Plants de Pommes de Terre (Rennes, France). Experimental conditions were two different regimes of night and day temperature conditions in the NS2-Rotoplan: temperate cold (15 to 24°C) and tropical hot (24 to 30°C). Experiments in the NS3-Rotoplan were conducted in tropical hot temperature conditions. Strains ($n = 156$) were inoculated to potato plants to assess their phenotype (Tables 2 and 3).

Pathogenicity assay of brown rot ecotype strains. Comparative analysis of virulence and aggressiveness was based on strains ($n = 14$) (Table 4) described above as potato pathogenic: cold-tolerant brown rot strains phylotype IIB-1, African brown rot strains phylotype III (24), and reference strains from phylotype I. Test were performed on tomato (*Solanum lycopersicon*) and on eggplant (*S. melongena*) carrying different levels of resistance to BW: tomato L390 is highly susceptible to *R. solanacearum* (33) and, thus, strains that wilt this tomato were considered virulent on *Solanaceae* spp.; eggplant MM960 is moderately resistant to BW (3) and tomato Hawaii7996 is highly resistant (5,33,35,39). These plants were grown in temperate cold and tropical hot temperature conditions in NS2-Rotoplan.

Pathogenicity assay of phylotype IIB. The phenotype of strains ($n = 29$) (Table 5) clustered into brown rot IIB-1 and IIB-2; Moko disease IIA-6, IIB-3, and IIB-4; and blood disease bacterium IV-10 lineages were used to challenge on L390, MM960, Hawaii7996 ($n = 10$ plants), and Cavendish banana cv. Grande Naine ($n = 5$ plants) to established virulence pattern. Infected plants were placed in tropical hot temperature conditions in NS3-Rotoplan.

Statistics. Pathogenicity was estimated by using a colonization index (CI) (28) based on wilted plants and latent infections: $CI = N_{WP} + (N_S \times R_S)$, where N_{WP} is the percentage of wilted plants, N_S is the percentage of symptomless plants, and R_S is the percentage of those asymptomatic plants sampled from which *R. solanacearum* was recovered. Data from pathogenicity tests were analyzed with R software 2.9.2 (31) and its specific libraries *cluster*, *MASS*, and *fpc*. The purpose of the following clustering method was to characterize the variability of phenotypes and, thus, identify clusters of strains that shared similar virulence or aggressiveness patterns. We used two methods to discriminate these clusters: (i) an agglomerative nesting which produced an ascendant hierarchical classification tree using Manhattan distance, where the validation of clusters is based on the internode distances, and (ii) a fuzzy analysis clustering which produced a partitioning, where the validation of the number of clusters was assessed by calculating the corrected Rand index between two clusterings with k and $k + 1$ clusters. A Rand index close to one validates the smallest clustering. Classification and partitioning were checked with biological data for supporting meaningful clusters.

RESULTS

Genetic diversity and phylogeny. Based on the PCR approach, strains of *R. solanacearum* used in this study ($n = 210$) (Table 1) clustered into phylotype I ($n = 2$; 1%), phylotype II ($n = 197$; 93.8%), phylotype III ($n = 8$; 3.8%), and phylotype IV ($n = 3$; 1.4%). From this collection, 106 strains (50.5%) originated or were intercepted in EuMr. Among the 15 different hosts of origin, potato and tomato were predominant, with 51% ($n = 107$) and 22% ($n = 46$) of the strains, respectively. The initial NJ tree computed from *egl* sequences was composed of the subset of 129 newly obtained sequences associated with 57 reference sequences representing all phylotypes; however, because all brown-rot strains in phylotype IIB-1 produced identical partial *egl* sequences, only 7 representatives were included to compute the phylogenetic tree (Fig. 1). Strains isolated from potato ($n = 84$) were distributed among all four phylotypes. A majority of strains from EuMr ($n = 71$) were isolated from potato ($n = 57$). The *egl* sequences of 94 of 129 strains clustered into phylotype IIB-1, including 66 sequences from strains originating from EuMr and 75 sequences from strains isolated from potato. Only five strains originating from EuMr were not phylotype IIB-1: these strains clustered into phylotype IIA-7 with the tomato strain CFBP4822 from Finland, phylotype IIA-35 with the potato strain PD1100 from Egypt, phylotype IIB-3 with the potato strain UW28 from

Cyprus (7), phylotype IIB-4NPB with the tomato strain LNPV24.25 from France, and phylotype IIB-25 with the potato strain UQRS607 from Iran.

Pathogenicity assay of *R. solanacearum*. Strains of *R. solanacearum* inoculated to potato distributed into two clusters validated by the Rand index when considering separate temperature conditions. However, when considering pathogenicity scoring at both temperature conditions, the Rand index validated the distribution of strains into three clusters (Table 2). Cluster A strains ($n = 28$) were highly aggressive regardless of the incubation temperature and were all assigned into phylotype IIB-1 that belongs to cluster A (68% isolated from potato and 54% originating from EuMr). Cluster B strains ($n = 24$) were highly aggressive under tropical hot conditions but weakly aggressive (developing few or no wilted plants) under temperate cold temperatures. Strains in this group belonged to phylotypes I, IIA, IIB-1, III, and IV (58% isolated from potato and 27% originating from EuMr). Cluster C strains ($n = 35$) induced little or no wilt symptoms regardless of the incubation temperature. In total, 71%

of strains from Cluster C were isolated from potato (57% originating from EuMr), of which 69% were phylotype IIB-1 and other were representatives of phylotypes I, IIA, III, and IV. In this experiment, certain strains did not produce BW under either tropical hot or temperate cold conditions, although these strains were able to establish latent infections in potato ($n = 6$). These strains were representative of phylotypes IIB-1 ($n = 5$) and IIB-26 (CIP240).

Strains not pathogenic to potato ($n = 20$; $CI = 0$) clustered into all phylotypes; namely, I-45, IIA-7, IIB-1 ($n = 15$), III-19, III-20, and IV-9. Strains not detected at Reunion (exotic) that were tested in NS3-Rotoplan ($n = 69$) under tropical hot temperature conditions distributed into three clusters validated by the Rand index (Table 3). Cluster A strains ($n = 32$; $CI \geq 90$) were highly aggressive and distributed in phylotypes IIB-1, IIB-2, IIB-4, III (CMR20), and IV. Most of these strains were isolated from potato (50%) and originated from EuMr (37.5%). Cluster B strains were moderately aggressive ($n = 20$; $80 \geq CI \geq 50$) and distributed in phylotypes IIA, IIB, III, and IV. Among these strains, 45% were

TABLE 2. Pathogenicity to potato of *Ralstonia solanacearum* strains clustered into phylotypes reported at Reunion (nonexotic strains), grown under different temperatures in security level NS2-Rotoplan^a

Strain	Origin, host	Phylotype	Hot ^b			Cold ^c			Global ^d
			w	CI	Cluster	w	CI	Cluster	
PSS4	Taiwan, tomato	I-15	9	90	A	3	30	C	B
GMI1000	French Guiana, tomato	I-18	8	80	A	0	30	C	B
CIP365	Philippines, potato	I-45	0	0	C	0	0	C	C
JS927	Porto Rico, tomato	IIA-7	0	0	C	0	0	C	C
JS967	Kenya, potato	IIA-7	2	20	C	0	0	C	C
CFBP4613	Brazil, potato	IIA-28	4	40	C	0	0	C	B
CFBP3105	Peru, potato	IIA-39	4	40	C	0	0	C	B
UW469	Brazil, potato	IIA-40	5	50	A	0	0	C	B
CFBP1417	Australia, potato	IIB-1	4	90	A	4	70	A	A
CFBP1810	Haiti, potato	IIB-1	8	80	A	6	90	A	A
CFBP3579	France, tomato	IIB-1	0	0	C	0	0	C	C
CFBP3583	Egypt, potato	IIB-1	0	10	C	0	0	C	C
CFBP3784	Portugal, potato	IIB-1	1	20	C	1	30	C	B
CFBP3785	Portugal, potato	IIB-1	8	80	A	3	30	C	B
CFBP3857	Netherlands, potato	IIB-1	2	20	C	0	0	C	C
CFBP3858	Netherlands, potato	IIB-1	7	70	A	2	60	A	B
CFBP3863	Morocco, potato	IIB-1	10	100	A	6	90	A	A
CFBP3865	France, potato	IIB-1	0	0	C	0	0	C	C
CFBP3870	South Africa, potato	IIB-1	3	50	A	2	30	C	B
CFBP3884	Sweden, potato	IIB-1	0	0	C	0	0	C	C
CFBP3886	Belgium, potato	IIB-1	4	40	C	1	10	C	B
CFBP3927	Greece, potato	IIB-1	0	0	C	0	0	C	C
CFBP3937	France, bittersweet	IIB-1	10	100	A	9	90	A	A
CFBP4577	Egypt, potato	IIB-1	0	0	C	0	0	C	C
CFBP4578	Egypt, tomato	IIB-1	5	60	A	2	30	C	B
CFBP4583	Egypt, potato	IIB-1	10	100	A	10	100	A	A
CFBP4585	Turkey, potato	IIB-1	10	100	A	9	100	A	A
CFBP4587	United Kingdom, potato	IIB-1	10	100	A	10	100	A	A
CFBP4588	United Kingdom, potato	IIB-1	4	60	A	3	70	A	B
CFBP4597	United Kingdom, tomato	IIB-1	10	100	A	10	100	A	A
CFBP4598	Cyprus, potato	IIB-1	1	10	C	0	0	C	C
CFBP4599	Sweden, potato	IIB-1	8	80	A	8	80	A	A
CFBP4600	Sweden, bittersweet	IIB-1	10	100	A	8	100	A	A
CFBP4604	Netherlands, potato	IIB-1	10	100	A	9	100	A	A
CFBP4609	Slovenia, potato	IIB-1	0	0	C	0	0	C	C
CFBP4634	Spain, potato	IIB-1	2	20	C	2	20	C	C
CFBP4652	France, tomato	IIB-1	7	70	A	3	50	A	B
CFBP4787	Portugal, potato	IIB-1	7	70	A	8	100	A	A

(continued on next page)

^a Abbreviations: w = number of wilted plants out of 10 plants; colonization index (CI) = $N_{WP} + (N_S \times R_S)$, where N_{WP} = percentage of wilted plants, N_S = percentage of symptomless plants, and R_S = percentage of those asymptomatic plants sampled from which *R. solanacearum* was recovered; and Cluster = clusters based on CI computed after fuzzy analysis clustering. Rand index validated, in cold temperatures conditions, two clusters: A (CI ≥ 50) and B (CI ≤ 40) (2 vs. 3 = 0.634; 2 vs. 4 = 0.878; 3 vs. 4 = 0.878). Rand index validated, in hot tropical temperatures conditions, two clusters: A (CI ≥ 50) and B (CI ≤ 40) (2 vs. 3 = 0.594; 2 vs. 4 = 0.499; 3 vs. 4 = 0.557).

^b Hot conditions referred to a night and day temperatures of 24 and 30°C, respectively.

^c Cold conditions referred to a night and day temperatures of 15 and 24°C, respectively.

^d Global = clusters based on CI on both hot and cold temperatures computed after fuzzy analysis clustering. Rand index validated three clusters: A, B, and C. A Rand index close to 1 validates the smallest clustering (2 vs. 3 = 0.491; 2 vs. 4 = 0.491; 3 vs. 4 = 0.770).

members of phylotype IIB-1, 75% were isolated from potato, and 30% originated from EuMr. Cluster C strains ($n = 17$; $CI \leq 40$) were weakly or not aggressive. This cluster encompassed a majority of phylotype IIB-1 (47%) and strains in phylotype IIA and III. Most of these were strains isolated from potato (82%) and originated from EuMr (76%). Some strains in phylotypes IIA-7 (CFBP4822), IIB-1 ($n = 5$), IIB-3 (Molk2 and UW28), and III-21 were not able to develop wilt on potato under tropical hot experimental conditions but were able to establish latent infections. Strains not pathogenic to potato ($CI = 0$) in tropical hot experimental conditions were composed of phylotype IIB-1 (potato strains CFBP3873 from Belgium and CFBP4607 from Portugal) and Moko disease-causing IIB-4 strain CFBP1184 from Honduras.

Pathogenicity assay of brown rot ecotype strains. In the NS2-Rotoplan, a subset of strains ($n = 14$) tested under tropical hot and temperate cold temperatures pathogenic to potato distributed into two clusters validated by the Rand index (Table 4). An identical clustering result was obtained whether temperatures were statistically analyzed separately or not. Strains in cluster A

were all IIB-1 strains with high pathogenicity whatever the plant-temperature combinations, except the South African potato strain CFBP3870 assigned in cluster B. Strains in cluster B were weakly pathogenic under temperate cold temperature conditions compared with the tropical hot temperature conditions.

Pathogenicity assay of phylotype IIB. Under tropical hot temperature conditions, strains tested in NS3-Rotoplan ($n = 29$) distributed in three different clusters that were validated by the Rand index (Table 5). Cluster A strains ($n = 5$) were highly virulent and phylogenetically clustered into phylotypes IIB-1 and IIB-4NPB. Cluster B strains ($n = 16$) were able to wilt or to establish latent infections on all tested hosts; namely, GMI1000 (phylotype I-18) and Moko disease-causing strains from phylotypes IIA-6, IIB-3, and IIB-4. Cluster C strains ($n = 8$) caused no or little wilt or strains that were able to establish latent infections on susceptible tomato L390. Cluster C included strains from phylotypes IIA-6, IIB-2, IIB-3, and IIB-4 but also from phylotypes IIB-25 and IV-10. Two strains, CFBP1410 and CFBP1416, in phylotypes IIB-2 and IIB-3, respectively, were not able to develop wilt in tropical hot temperature conditions but did establish latent

TABLE 2. (continued from preceding page)

Strain	Origin, host	Phylotype	Hot ^b			Cold ^c			Global ^d
			w	CI	Cluster	w	CI	Cluster	
CFBP4788	France, tomato	IIB-1	0	0	C	0	0	C	C
CFBP4801	Reunion, tomato	IIB-1	9	90	A	8	80	A	A
CFBP4808	Israel, potato	IIB-1	0	0	C	0	0	C	C
CFBP4811	France, Black nightshade	IIB-1	0	0	C	0	0	C	C
CFBP4812	France, tomato	IIB-1	0	0	C	0	0	C	C
CFBP4821	Sweden, potato	IIB-1	0	0	C	0	0	C	C
CFBP7101	Spain, potato	IIB-1	0	0	C	0	20	C	C
CFBP7105	Spain, water (river)	IIB-1	0	10	C	0	0	C	C
CFBP7108	Kenya, <i>Pelargonium</i> sp.	IIB-1	4	60	A	0	70	A	B
CMR34	Cameroon, tomato	IIB-1	8	90	A	9	100	A	A
CMR44	Cameroon, potato	IIB-1	10	100	A	10	100	A	A
GRISP2335D	France, potato	IIB-1	10	100	A	9	100	A	A
GRISP2336B	France, tomato	IIB-1	9	90	A	9	100	A	A
IPO1609	Netherlands, potato	IIB-1	1	10	C	0	0	C	C
JQ1006	Reunion, potato	IIB-1	9	100	A	0	0	C	B
JQ1019	Reunion, potato	IIB-1	5	60	A	4	50	A	B
JQ1023	Reunion, tomato	IIB-1	3	40	C	1	30	C	B
JQ1051	Reunion, tomato	IIB-1	0	0	C	0	0	C	C
JQ1073	Reunion, tomato	IIB-1	10	100	A	9	100	A	A
JQ1107	Reunion, potato	IIB-1	10	100	A	10	100	A	A
JQ1131	Reunion, potato	IIB-1	8	90	A	7	80	A	A
JS925	Sri Lanka, potato	IIB-1	6	60	A	10	100	A	A
JS926	India, potato	IIB-1	0	20	C	0	0	C	C
JT511	Reunion, potato	IIB-1	8	80	A	4	60	A	B
JT516	Reunion, potato	IIB-1	1	30	C	0	40	C	B
JT646	Sri Lanka, potato	IIB-1	0	0	C	0	0	C	C
LMG17139	Cyprus, potato	IIB-1	10	100	A	8	100	A	A
LNPV19.66	France, potato	IIB-1	10	100	A	7	80	A	A
LNPV23.54	France, potato	IIB-1	0	0	C	0	0	C	C
LNPV27.43	Guadeloupe, anthurium	IIB-1	0	0	C	0	0	C	C
LNPV28.23	Reunion, potato	IIB-1	10	100	A	10	100	A	A
LNPV28.49	France, bittersweet	IIB-1	10	100	A	8	90	A	A
PD441	Sweden, potato	IIB-1	0	10	C	0	10	C	C
PSS525	Taiwan, potato	IIB-1	8	100	A	10	100	A	A
RTG2	Guinea, potato	IIB-1	9	100	A	6	100	A	A
UW551	Kenya, <i>Pelargonium</i> sp.	IIB-1	10	100	A	7	90	A	A
CIP240	Brazil, potato	IIB-26	0	20	C	0	0	C	C
JT525	Reunion, <i>Pelargonium</i> sp.	III-19	0	0	C	0	0	C	C
MAD-29	Madagascar, potato	III-19	2	20	C	0	0	C	C
CMR33	Cameroon, tomato	III-20	3	30	C	0	20	C	B
J25	Kenya, potato	III-20	0	0	C	0	0	C	C
CFBP3059	Burkina Faso, eggplant	III-23	7	80	A	0	0	C	B
CMR32	Cameroon, huckleberry	III-29	3	40	C	1	20	C	B
DGBBC1227	Guinea, potato	III-42	10	100	A	0	10	C	B
DGBBC1125	Guinea, potato	III-43	1	20	C	0	0	C	C
DGBBC1138	Guinea, potato	III-43	7	70	A	0	20	C	B
CMR66	Cameroon, huckleberry	III-49	6	60	A	0	0	C	B
MAFF301552	Japan, tomato	IV-8	4	50	A	0	10	C	B
R28	Indonesia, clove	IV-9	0	0	C	0	0	C	C

TABLE 3. Pathogenicity to potato of *Ralstonia solanacearum* strains clustered into phylotypes not reported at Reunion (exotic strains), grown under tropical hot 24 and 30°C (night and day, respectively) temperatures in security level NS3-Rotoplan

Strain	Origin, host	Phylotype	w ^a	CI ^b	Cluster ^c
RUN394	Grenada, banana Bluggoe	IIA-6	4	60	B
CFBP4822	Finland, tomato	IIA-7	0	10	C
ICMP7963	Kenya, potato	IIA-7	5	70	B
CIP301	Peru, potato	IIA-35	2	40	C
IBSBF1546	Brazil, California fuchsia	IIA-35	9	100	A
PD1100	Egypt, potato	IIA-35	3	40	C
CFBP2968	Reunion, potato	IIA-39	7	80	B
AP31H	Uruguay, potato	IIB-1	5	70	B
CFBP3582	Egypt, potato	IIB-1	0	50	B
CFBP3872	Belgium, potato	IIB-1	7	80	B
CFBP3873	Belgium, potato	IIB-1	0	0	C
CFBP3874	Belgium, potato	IIB-1	10	100	A
CFBP3876	Belgium, potato	IIB-1	1	20	C
CFBP3885	Belgium, potato	IIB-1	0	20	C
CFBP3889	Spain, potato	IIB-1	0	40	C
CFBP4580	Egypt, potato	IIB-1	9	100	A
CFBP4581	Egypt, potato	IIB-1	9	100	A
CFBP4582	Egypt, potato	IIB-1	9	100	A
CFBP4584	Egypt, potato	IIB-1	10	100	A
CFBP4601	Belgium, potato	IIB-1	1	10	C
CFBP4605	Netherlands, potato	IIB-1	9	90	A
CFBP4607	Portugal, potato	IIB-1	0	0	C
CFBP4608	Portugal, potato	IIB-1	0	30	C
CFBP4635	Spain, potato	IIB-1	9	100	A
CFBP4823	Egypt, potato	IIB-1	4	60	B
CFBP7102	Spain, potato	IIB-1	5	70	B
CFBP7103	Spain, potato	IIB-1	10	100	A
CFBP7104	Spain, potato	IIB-1	8	80	B
DGBBC1181	Belgium, potato	IIB-1	4	50	B
ETAC	Uruguay, potato	IIB-1	3	50	B
JS976	Spain, potato	IIB-1	10	100	A
JS977	Spain, potato	IIB-1	10	100	A
LMG2300	Israel, potato	IIB-1	0	40	C
LMG9576	Belgium, potato	IIB-1	8	90	A
PO41	China, potato	IIB-1	4	90	A
RM	Uruguay, potato	IIB-1	10	100	A
UW394	South Africa, potato	IIB-1	5	70	B
CFBP1410	Colombia, banana plantain	IIB-2	9	100	A
CFBP3879	Colombia, potato	IIB-2	4	70	B
CFBP4611	Colombia, potato	IIB-2	3	80	B
CIP418	Indonesia, peanut	IIB-3	5	70	B
Molk2	Philippines, <i>Musa</i> sp.	IIB-3	0	40	C
UW28	Cyprus, potato	IIB-3	0	40	C
CFBP1184	Honduras, <i>Musa</i> sp.	IIB-4	0	0	C
LNPV31.10	French Guiana, <i>Musa</i> sp.	IIB-4	9	100	A
LNPV32.36	French Guiana, <i>Musa</i> sp.	IIB-4	7	90	A
LNPV32.37	French Guiana, banana plantain	IIB-4	9	90	A
LNPV32.40	French Guiana, <i>Musa</i> sp.	IIB-4	9	90	A
UW162	Peru, banana plantain	IIB-4	5	50	B
UW163	Peru, banana plantain	IIB-4	7	90	A
UW170	Colombia, <i>Heliconia</i> sp.	IIB-4	3	50	B
CIP10	Peru, potato	IIB-25	5	70	B
UQRS607	Iran, potato	IIB-25	2	20	C
NCPPB3987	Brazil, potato	IIB-28	3	100	A
CFBP7014	Trinidad, anthurium	IIB-51	9	100	A
CFBP6778	Martinique, tomato	IIB-4NPB	10	100	A
CFBP6783	Martinique, <i>Heliconia</i> sp.	IIB-4NPB	9	90	A
CFBP6797	Martinique, American nightshade	IIB-4NPB	10	100	A
JY200	Martinique, anthurium	IIB-4NPB	10	100	A
LNPV24.25	France, tomato	IIB-4NPB	9	100	A
RUN432	French Guiana, water (irrigation)	IIB-4NPB	10	100	A
NCPPB1018	Angola, potato	III-21	0	20	C
NCPPB332	Zimbabwe, potato	III-22	1	10	C
CMR15	Cameroon, tomato	III-29	2	50	B
CMR20	Cameroon, tomato	III-29	3	100	A
MAFF301558	Japan, potato	IV-8	8	90	A
CFBP6727	Indonesia, potato	IV-10	8	100	A
CFBP6728	Indonesia, potato	IV-10	6	80	B
PSI7	Indonesia, tomato	IV-10	10	100	A

^a Number of wilted plants out of 10 plants.

^b Colonization index (CI) = $N_{WP} + (N_S \times R_S)$, where N_{WP} = percentage of wilted plants, N_S = percentage of symptomless plants, and R_S = percentage of those asymptomatic plants sampled from which *R. solanacearum* was recovered.

^c Cluster = clustering based on the CI and obtained by a fuzzy analysis clustering. Rand index validated three clusters: A ($CI \geq 90$), B ($80 \geq CI \geq 50$), and C ($CI \leq 40$). A Rand index close to 1 validates the smallest clustering (2 vs. 3 = 0.484; 2 vs. 4 = 0.514; 3 vs. 4 = 0.811).

infections in plants. Nonpathogenic strains to both tomato and eggplant ($n = 4$) in tropical hot experimental conditions were phylogenetically clustered into phylotypes IIA-6, IIB-3, and IV-10. Strains pathogenic to banana were members of Moko phylotypes IIA-6, IIB-3, and IIB-4 and BDB phylotype IV-10 lineages. Strain CIP418 (phylotype IIB-3) isolated from peanut in Indonesia was virulent to banana and strain UW28 (phylotype IIB-3) isolated from potato in Indonesia Cyprus was not pathogenic on both host plants. All strains in Moko lineages established infection in banana plants (Table 5). Strains in phylotype IIB-4NPB were not able to infect banana. From all non-Moko disease-causing strains tested in phylotype IIB, all were able to establish latent infection in banana, with the exception of members of phylotypes IIB-25, IIB-26, IIB-28, and IIB-51. Strain GMI1000 showed no pathogenicity to banana.

DISCUSSION

In this study, we assessed genetic diversity, phylogeny, and pathogenicity traits challenging different *Solanaceae* spp. and banana with a set of strains of *R. solanacearum* from EuMr. These strains were mainly isolated from diseased potato plants from the field or quarantined by national plant health inspection services. Results showed a greater diversity than expected from the literature, especially regarding host range and pathotype of potato strains that clustered into phylotype II (10).

From the phylogenetic analysis of partial *egl* gene sequences and from the organismal phylogeny (16), it was evident that strains from *R. solanacearum* species complex phylotype II encompassed strains with both broad and narrow host ranges (11). Strains showing the narrowest host range belonged to ecotypes Moko (banana and heliconia) and brown rot (potato and tomato). These ecotypes have a much narrower definition reflected in their genetic diversity as well-described sequevars (IIB-1, IIB-2, IIA-6, IIA-24, IIB-3, and IIB-4), whereas broad host range strains are scattered among phylotype II. Based on the phylogenetic tree presented in this study, strains that cause BW on *Solanaceae* spp. distributed in all phylotypes (I, IIA, IIB, III, and IV), just as potato-pathogenic strains showed a wide range of aggressiveness

and virulence (Table 6), mirroring a wide genetic basis for pathogenicity on these hosts. Most remarkable were virulence patterns of strains clustering into the Moko disease-causing phylotypes IIB-3 and IIB-4; although isolated from *Musa* spp. and pathogenic to banana, most strains were virulent to susceptible potato and tomato cultivars but not to eggplant, and phylotype IIB-4 strains were found to be virulent on resistant tomato Hawaii7996. The Moko disease-causing phylotype IIA-6, RUN394, was the only strain virulent to tomato, compared with the two other strains of the same phylotype in the time course of the experiment. Thus, in our experimental conditions, most banana strains inoculated to susceptible potato and tomato clustered into same pathogenicity cluster along with GMI1000, the model strain virulent on a wide range of *Solanaceae* spp. (32), including Molk2, the sequenced model genome of Moko disease-causing IIB-3 strains (15). As expected, the BDB strain R229 that clustered into the phylotype IV-10 was only pathogenic to banana.

It is noteworthy that experimental conditions applied in these experiments (injured roots and high concentration of inoculum) were in favor of BW development. Nevertheless, some Moko disease-causing strains were able to neither establish latent infection on *Solanaceae* spp. nor develop wilt. To our knowledge, no Moko disease-causing strains have ever been isolated from a wilted tomato or potato in the field, although it is now apparent that they were able to establish infections in these hosts. The three exceptions in our study were the phylotype IIB-2 strain CFBP1410 isolated from plantain (cooking banana) in Colombia, which established latent infection into banana; the phylotype IIB-3 strain CIP418 isolated from peanut in Indonesia that wilted banana; and the phylotype IIB-3 strain UW28 isolated from potato that lacked pathogenicity on all plant species tested but still clustered in a Moko disease-causing phylotype.

As reported by Fegan and Prior (11), the polyphyletic nature of Moko disease-causing strains of *R. solanacearum* is evidenced by the observation that strains are found within phylotype IIA (sequevar 6) and phylotype IIB (sequevars 3 and 4). All Moko disease-causing strains in this study that were no more pathogenic to banana also lacked pathogenicity to tomato. This suggests a loss of major determinants in mechanisms governing patho-

TABLE 4. Pathogenicity to tomato and eggplant of nonexotic strains of *Ralstonia solanacearum*, grown under different temperatures in security level NS2-Rotoplan^a

Strain	Phylotype	Hot ^b								Cold ^c							
		L390		Hawaii7996		MM960		Clu		L390		Hawaii7996		MM960		Clu	Glob ^d
		w	CI	w	CI	w	CI			w	CI	w	CI	w	CI		
PSS4	I-15	10/8	100/90	5/0	50/10	10/0	100/80	A/B		4/8	50/80	0/0	0/0	1/0	20/30	B/B	B/B
GMI1000	I-18	10/10	100/100	3/0	50/20	0/0	0/0	B/B		7/0	70/10	0/0	20/0	0/0	0/10	B/B	B/B
CFBP3858	IIB-1	10/10	100/100	8/8	80/100	6/3	60/80	A/A		9/6	100/90	8/0	80/70	3/0	70/50	A/A	A/A
CFBP3870	IIB-1	7/9	70/100	0/2	0/100	1/0	10/60	B/A		2/3	20/50	1/0	10/10	5/0	50/20	B/B	B/B
CFBP4787	IIB-1	10/10	100/100	8/7	90/100	8/1	80/70	A/A		9/10	100/100	10/3	100/60	9/0	100/70	A/A	A/A
CMR34	IIB-1	10/10	100/100	10/3	100/90	7/0	70/70	A/A		10/10	100/100	9/2	100/70	8/0	80/50	A/A	A/A
JT516	IIB-1	6/9	80/100	9/5	90/80	5/0	60/50	A/A		5/8	60/90	8/0	90/40	6/0	70/40	A/A	A/A
LNPV28.23	IIB-1	10/10	100/100	10/4	100/80	9/0	90/80	A/A		10/10	100/100	9/5	90/80	9/0	90/80	A/A	A/A
PSS525	IIB-1	10/10	100/100	9/5	100/100	6/1	70/80	A/A		8/10	90/100	8/0	90/80	5/0	80/60	A/A	A/A
UW551	IIB-1	10/10	100/100	3/2	100/100	5/0	70/90	A/A		8/9	90/90	8/5	100/70	7/0	80/40	A/A	A/A
CMR33	III-20	7/0	70/10	0/0	0/0	0/0	0/10	B/B		3/0	30/20	0/0	10/10	0/0	0/10	B/B	B/B
CFBP3059	III-23	10/7	100/100	10/0	100/20	0/0	0/40	A/B		8/0	80/30	0/0	0/20	1/0	20/20	B/B	B/B
DGBBC1138	III-43	10/0	100/30	0/0	0/10	3/0	30/30	B/B		1/1	20/70	0/0	0/0	1/0	10/0	B/B	B/B
CMR66	III-49	8/9	80/100	0/0	10/20	0/0	0/10	B/B		5/1	50/40	0/0	10/0	0/0	0/10	B/B	B/B

^a Abbreviations: w = number of wilted plants out of 10 plants; colonization index (CI) = $N_{WP} + (N_S \times R_S)$, where N_{WP} = percentage of wilted plants, N_S = percentage of symptomless plants, and R_S = percentage of those asymptomatic plants sampled from which *R. solanacearum* was recovered; and Clu = clustering based on the CI and obtained by a fuzzy analysis clustering. Data organized as follows: replicate 1/replicate 2.

^b Hot conditions referred to a night and day temperatures of 24 and 30°C, respectively. Rand index validated, at hot tropical temperatures, two clusters: A and B (2 vs. 3 = 0.715; 2 vs. 4 = 0.367; 3 vs. 4 = 0.594; second replicate: 2 vs. 3 = 0.822; 2 vs. 4 = 0.455; 3 vs. 4 = 0.594).

^c Cold conditions referred to a night and day temperature of 15 and 24°C, respectively. Rand index validated, at cold temperatures, two clusters: A and B (2 vs. 3 = 0.729; 2 vs. 4 = 0.502; 3 vs. 4 = 0.502; second replicate: 2 vs. 3 = 0.775; 2 vs. 4 = 0.518; 3 vs. 4 = 0.518).

^d Glob = global clusters based on CI on both hot and cold temperatures computed after fuzzy analysis clustering. Rand index validated two clusters: A and B. A Rand index close to 1 validates the smallest clustering (2 vs. 3 = 0.729; 2 vs. 4 = 0.495; 3 vs. 4 = 0.728; second replicate: 2 vs. 3 = 0.729; 2 vs. 4 = 0.309; 3 vs. 4 = 0.391). Data organized as follows: replicate 1/replicate 2.

genicity for both the broad and narrow host range. Because we demonstrated that most strains pathogenic to banana were also pathogenic to potato and tomato, it is possible that other non-Moko disease-causing strains in each subcluster might have lost the ability to cause wilt on banana. Alternatively, horizontal gene transfer may have taken place between these two *R. solanacearum* lineages (IIA and IIB), allowing a non-banana-pathogenic organism to become a banana pathogen. In addition, some strains that were not isolated from any *Musaceae* maintained the ability to establish latent infections on banana, especially brown rot phylotype IIB-1 and IIB-2 strains and strain IBSBF1712 (IIB-27) isolated from pelargonium. Latent infections (i.e., the capacity of a strain to establish itself into the vascular tissues on a host [asymptomatic plant]) evidenced the conservation of an early step mechanism required for pathogenicity. When established as latent infections in a host, *R. solanacearum* strains may develop wilt or not (34), turning plant vasculature into an ecological niche of choice. On the one hand, from a phylogenetic point of view, closely related sequevars IIB-1, IIB-3, and IIB-4 address the question of host adaptation mechanisms which may have undergone a similar bottleneck evolution. On the other hand, from an epidemiological point of view and as regards actual cultural practice, IIB-1 brown rot strains that adapted to host cropped under a highland cold environment have almost no chance to meet with extensive tropical lowland crops, such as banana. In other words, a major finding of this work was to improve our understanding of the phenotype of *R. solanacearum* strains that are genetically and phylogenetically well characterized. A better understanding of pathogenicity traits, differentiating a nonhost versus susceptible or asymptomatic host, provides a relevant

frame to accurately approach underlying mechanisms for host speciation at the “omics” era.

Pathogenicity assessment of brown rot strains was congruent with the literature (25) and highlighted the fact that only phylo-type IIB-1 strains maintain a high level of virulence regardless of temperature tested. Other strains, including the most closely related phylotype, IIB-2, and the phylogenetically distant African brown rot strains from phylotype III (24), although isolated from highland areas, dramatically decreased in virulence under cold temperatures. This indicates that the virulence factors for cold tolerance need further studies. In addition, because all IIB-1 strains that lack pathogenicity to potato under cold temperature were also weakly or not pathogenic under a tropical hot environment, it is anticipated that pathogenicity may have been altered in these strains. As expected from the literature, cold-tolerant EuMr strains isolated from wilted potato, tomato, or bittersweet were clonal and phylogenetically clustered into phylotype IIB-1, equivalent to R3bv2. Nonetheless, according to pathogenicity tests on *Solanaceae* spp., phenotypes statistically differed into different pathogenicity clusters. Since their first introduction decades ago in Europe, brown rot strains may have undergone genomic adaptation in local populations and were selected according to their fitness.

In our study, 101 of 106 strains originating or being intercepted in EuMr clustered into the brown rot phylotype IIB-1. Within the phylotype IIB-4NPB, we identified strain LNPV24.25 isolated in 2001 from a wilted tomato produced in a greenhouse in the southeast of France (Laboratoire National de la Protection des Végétaux, Angers, *personal communication*), which indicates, to our knowledge, the first report of phylotype IIB-4NPB strain in

TABLE 5. Pathogenicity to tomato, eggplant and banana of exotic strains of *Ralstonia solanacearum* grown under tropical hot 24/30°C (night/day) temperatures in security level NS3-Rotoplan^a

Strain	Origin, host	Phylotype	L390 ^b		Hawaii7996 ^b		MM960 ^b		Cluster ^c	Banana ^d	
			w	CI	w	CI	w	CI		w	CI
GMI1000	French Guiana, tomato	I-18	7 (10)	70	1 (0)	10	0 (0)	0	B	0	0
GMI8044	Grenada, banana	IIA-6	0 (0)	0	0 (0)	0	0 (0)	0	C	0	80
RUN394	Grenada, banana Bluggoe	IIA-6	6 (6)	90	0 (4)	50	0 (0)	20	B	5	100
UW181	Venezuela, banana plantain	IIA-6	0 (0)	0	0 (0)	0	0 (0)	0	C	0	100
CFBP2957	Martinique, tomato	IIA-36	6 (9)	80	0 (2)	20	4 (0)	60	B	0	100
CMR34	Cameroon, tomato	IIB-1	10 (8)	100	5 (7)	80	0 (0)	60	A	0	80
LNPV28.23	Reunion, potato	IIB-1	10 (10)	100	3 (8)	70	9 (5)	100	A	0	80
UW551	Kenya, <i>Pelargonium</i> sp.	IIB-1	10 (10)	100	0 (1)	40	4 (1)	100	A	0	100
CFBP1410	Colombia, banana plantain	IIB-2	0 (0)	30	0 (0)	20	0 (0)	50	B	0	100
CFBP3879	Colombia, potato	IIB-2	0 (0)	10	0 (0)	0	0 (0)	0	C	0	80
CFBP4611	Colombia, potato	IIB-2	0 (0)	10	0 (0)	0	0 (0)	0	C	0	80
CFBP1416	Costa Rica, banana plantain	IIB-3	0 (0)	60	0 (0)	20	0 (0)	0	B	0	100
CIP417	Philippines, banana	IIB-3	5 (3)	80	0 (0)	0	0 (0)	10	B	3	100
CIP418	Indonesia, peanut	IIB-3	2 (9)	30	0 (0)	10	0 (0)	0	C	5	100
Molk2	Philippines, <i>Musa</i> sp.	IIB-3	6 (8)	90	0 (0)	0	0 (0)	10	B	5	100
UW28	Cyprus, potato	IIB-3	0 (0)	0	0 (0)	0	0 (0)	0	C	0	0
UW9	Costa Rica, <i>Heliconia</i> sp.	IIB-3	0 (3)	50	0 (0)	10	0 (0)	10	B	0	100
UW160	Peru, banana plantain	IIB-4	9 (10)	90	4 (3)	60	0 (0)	0	B	3	100
UW163	Peru, banana plantain	IIB-4	9 (9)	90	4 (1)	60	0 (0)	10	B	5	100
CIP10	Peru, potato	IIB-25	2 (2)	40	0 (0)	0	0 (0)	0	C	0	0
IBSBF2001	Brazil, tomato	IIB-25	7 (9)	80	0 (5)	30	0 (3)	0	B	0	0
CIP240	Brazil, potato	IIB-26	5 (7)	60	0 (0)	40	0 (0)	40	B	0	0
IBSBF1712	Brazil, <i>Pelargonium</i> sp.	IIB-27	10 (4)	100	0 (0)	20	0 (0)	0	B	0	100
NCPBP3987	Brazil, potato	IIB-28	3 (3)	60	0 (0)	0	0 (0)	10	B	0	0
CFBP7014	Trinidad, anthurium	IIB-51	6 (5)	100	1 (0)	20	0 (0)	40	B	0	0
CFBP6783	Martinique, <i>Heliconia</i> sp.	IIB-4NPB	10 (10)	100	6 (7)	70	7 (7)	80	A	0	0
IBSBF1503	Brazil, cucumber	IIB-4NPB	10 (10)	100	8 (8)	90	7 (6)	80	A	0	0
LNPV24.25	France, tomato	IIB-4NPB	10 (9)	100	5 (9)	50	2 (2)	20	B	0	0
R229	Indonesia, banana	IV-10	0 (0)	0	0 (0)	0	0 (0)	0	C	5	100

^a Colonization index (CI) = $N_{WP} + (N_S \times R_S)$, where N_{WP} = percentage of wilted plants, N_S = percentage of symptomless plants, and R_S = percentage of those asymptomatic plants sampled from which *R. solanacearum* was recovered.

^b Number of wilted plants (w) out of 10 plants. Numbers in parentheses were scores obtained in an additional experiment (replicate) without investigating latent infection (no plant wilted but successful recovery of *R. solanacearum* on at least one plant).

^c Cluster = phenotypic groups based on the CI and obtained by a fuzzy analysis clustering. Rand index validated three clusters: A, B, and C. A Rand index close to 1 validates the smallest clustering (2 vs. 3 = 0.186; 2 vs. 4 = 0.413; 3 vs. 4 = 0.559).

^d Number of wilted plants (w) out of five plants.

France; however, the infection was contained and no outbreak has been surveyed during the past decade. These strains assigned to phylotype IIB-4NPB, reported as emergent in Martinique by Wicker et al. in the French West Indies (40), are phylogenetically undistinguishable from the Moko disease-causing phylotype IIB-4 strains by partial *egl* gene analyses. Phylotypes IIB-4NPB strains were highly virulent and aggressive and overcame resistance on *Solanaceae* spp. They were statistically clustered in the same pathogenicity group as the brown rot phylotype IIB-1 strains and were not pathogenic to banana. The origin of this emerging pathogen remains unsolved and its pathogenicity toward cultivated

Musa spp. may have been lost or never acquired (41). In addition to LNPV24.25 (IIB-4NPB), four others strains from EuMr did not cluster into phylotype IIB-1: (i) potato strain UW28 (Cyprus) clustered into the Moko disease-causing phylotype IIB-3, equivalent to the multilocus genotype group 24 (MLG24), reported by Cook and Sequeira (7); (ii) tomato strain CFBP4822 (Finland) clustered into the American phylotype IIB-7; (iii) potato strain PD1100 (Finland) clustered into the phylotype IIA-35; and (iv) potato strain UQRS607 (Iran) that was previously reported as phylotype IIB-25 (26). These strains showed that, despite the great proportion of brown rot phylotype IIB-1 strains in EuMr

Fig. 1. Phylogenetic neighbor-joining tree based on partial sequences of the endoglucanase (*egl*) gene sequences of a subset of strains from different bacterial collections or intercepted by national plant health inspection services (in bold), and *Ralstonia solanacearum* species complex reference strains. The number at each node is the bootstrap value (2,000 resamplings), and only significant bootstrap values >80% are indicated. Scale bar represents 1 nucleotide substitution per 100.

TABLE 6. Summary of the temperature-related virulence on potato of phylogenetic groups of *Ralstonia solanacearum*^a

Phylogenetic groups ^b	Hot	Cold
I	+	–
IIA	±	–
IIA Moko	+	nd
IIB-1 Brown rot	+	+
IIB Moko	+	nd
III	+	–
IV	+	–
IV (BDB)	–	nd
IV (RSY)	–	–

^a Virulence observed on potato at tropical hot (24 and 30°C, night and day, respectively) and temperate cold (15 and 24°C, night and day, respectively) temperatures; + = most of the strains tested (≥50%) are virulent; – = all strains tested are not virulent; ± = some of the strains tested are virulent; nd = not done.

^b BDB = blood disease bacterium and RSY = *Ralstonia syzygii*.

territory, other phylotypes may represent a potential threat to *Solanaceae* production. We also detected brown rot phylotype IIB-1 strains isolated from Anthurium and potato in Guadeloupe (French West Indies): LNPV27.43 and CFBP3880, respectively. Because R3bv2 was not reported from Guadeloupe (30), this suggests that these strains may be recently introduced.

In addition to deciphering pathogenicity traits, we described a set of three genetic resources that are useful in identifying pathogenicity clusters within *R. solanacearum* strains; namely, BW-susceptible tomato line L390, moderately resistant eggplant MM960, and highly resistant tomato Hawaii7996. Considering these three hosts, results showed that IIB-1 strains distributed into statistical clusters that gathered highly pathogenic and pathogenic phenotypes of strains according to their virulence and aggressiveness. This confirms and extends a previous report showing that no immunity or complete resistance was observed in any accessions in the *Lycopersicon* genus and relatives, facing IIB-1 strain JT516 (6).

These findings revealed that phylotype II strains share more virulence traits than expected from the literature, which is of major concern for investigating bacterial factors that control host or nonhost specificity, especially through post-genomic analysis of the *R. solanacearum* species complex.

ACKNOWLEDGMENTS

This work was funded by the Fédération Nationale des Producteurs de Plants de Pommes de Terre, Mission-DAR, Grant-7124 of the French Ministry of Food, Agriculture and Fisheries. The European Regional Development Fund (ERDF) of the European Union, Conseil Régional de La Réunion also provided financial support as part of Biorisk program developed at Centre de coopération Internationale en Recherche Agronomique pour le Développement. We thank M. Fegan, E. Wicker, J. C. De Cambiaire, and O. Pruvost for critical review before submission; F. Chiroleu for expertise in statistical analyses with R packages; J. M. Baptiste, J. J. Cheron, and S. Lebon for technical assistance; and institutions cited in the text for their courtesy in sharing strains of *Ralstonia solanacearum*.

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