Final Report

Project title (Acronym)
Rapid identification of plant-health related bacteria by MALDI-TOF mass spectrometry (MALD-ID)

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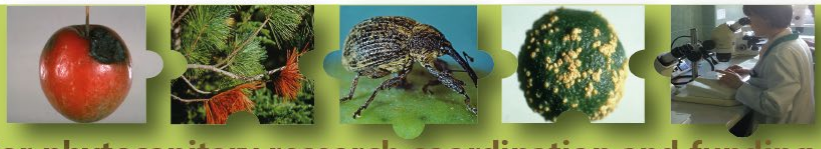
Contents

1. Research consortium partners 3
2. Short project report 4
2.1. Executive summary 4
2.2. Project aims 4
2.3. Description of the main activities 5
2.4. Main results 6
2.5. Conclusions and recommendations to policy makers 8
2.6. Benefits from trans-national cooperation 9
3. Publications 10
4. Open Euphresco data 11



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2. Short project report

2.1. Executive summary

The Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF) mass spectrometry is an analytical method that can be used as a diagnostic tool to identify microorganisms. The method is rapid (less than 1h from sample preparation to results) and accurate and it is widely used in other fields, such as human health and animal health.

Mass spectrometry allows to obtain a unique molecular (mainly proteins) fingerprint of the sample analyzed, the mass spectrum, that can be used to characterize and eventually identify the sample through comparison with reference spectra of known samples. The quality and completeness of the reference databases is crucial for an accurate and reliable identification. The commercial reference databases available to support MALDI-TOF mass spectrometry identification are not specialized for plant-pathogenic bacteria. This precludes the use of MALDI-TOF mass spectrometry for the identification of plant-pathogenic bacteria.

In this framework, the aim of the Euphresco project 2018-A-271 'Rapid identification of plant-health related bacteria by MALDI-TOF mass spectrometry (MALD-ID)' was i) to build a reference database that can support the identification of selected plant-pathogenic bacterial genera and ii) to evaluate the possibility to use the MALDI-TOF mass spectrometry method as a diagnostic tool. The project gathered three partners from JKI (Germany), NIVIP (The Netherlands), and INRAE (France). The project allowed to produce 117 reference spectra for the identification of bacterial strains from eight genera: *Clavibacter*, *Curtobacterium*, *Erwinia, Pantoea, Pectobacterium*, *Pseudomonas*, *Ralstonia*, *and Xylophilus.* These strains were chosen to represent the known diversity of each genus or to better distinguish between regulated and not regulated in a same genus. These reference spectra, compatible with the Bruker System, are freely available from a public repository on the following url: https://doi.org/10.57745/7OJNOO.The project also allowed to validate the use of MALDI-TOF mass spectrometry for the analysis of more strains from these genera and to determine the accuracy and reliability of the tests.

The project showed that MALDI-TOF mass spectrometry allows to correctly identify plant-pathogenic bacterial strains at the genus level in all cases. For *Erwinia*, *Pantoea*, *Ralstonia*, and *Xylophilus* MALDI-TOF mass spectrometry is even able to correctly identify strains at the species level. However, the method is unable to discriminate accurately at the intra-specific level in all cases. In plant-pathology, strain identification should often be performed at intra-specific level. Consequently, MALDI-TOF mass spectrometry alone is not sufficient for a complete diagnosis. However, MALDI-TOF mass spectrometry can reliably identify the genus of the isolates and can be used as a rapid first screening that can help to further choose the most relevant test for a precise identification.

2.2. Project aims

MALDI-TOF (Matrix Assisted Laser Desorption Ionisation - Time of Flight) mass spectrometry (MS) has become a high-throughput and rapid method for routine microorganism identification. This method is based on the ionization of intracellular proteins from an isolate and separation of the ions according to their mass-to-charge ratio (*m*/*z*). This results in a unique fingerprint of the isolate named mass spectrum. This mass spectrum is then compared with reference spectra, which permits to identify the isolate.

A crucial point of the mass spectrometry-based identification lies on the quality and completeness of databases of reference spectra. MALDI-TOF mass spectrometry is now widely used for diagnostics in human medicine. For this reason, the databases provided with

the MALDI-TOF devices by the two main providers (Bruker and BioMérieux) are clinically-oriented and the spectra of plant-pathogenic bacteria are underrepresented. Thus, the possibility to obtain an accurate identification of plant-pathogenic bacteria is impaired.

However, MALDI-TOF mass spectrometry has been proved to be useful for the identification of plant pathogenic bacteria. For example, the method has sufficient discriminatory power to allow to distinguish members of the genus *Clavibacter* from the related *Curtobacterium* genus, and to distinguish the different subspecies (now elevated as separate species) of *Clavibacter michiganensis* from each other (Zaluga *et al*. 2011). Thus, the development of in-house reference spectra allows to overcome the limitations of the commercial databases.

The objectives of the MALD-ID project were, firstly to produce in-house reference mass spectra of plant-pathogenic bacteria to supplement the commercial databases. And secondly, to evaluate the use of MALDI-TOF mass spectrometry for the diagnosis of plant-pathogenic bacteria. The project gathered partners from INRAE (France), JKI (Germany), NIVIP (The Netherlands) and ZHAW (Switzerland).

The project focused on a limited list of plant-pathogenic bacteria taxa (*Clavibacter*, *Curtobacterium*, *Erwinia*, *Pantoea*, *Pseudomonas syringae*, *Pectobacterium*, *Ralstonia* and *Xylophilus*) to which the partners had access to relevant biological resources. Then each partner worked to produce reference spectra for one or several taxa, following its expertise. Finally, some partners exchanged spectra to test the feasibility of data exchange.

2.3. Description of the main activities

2.3.1. Choice of technical solution for MALDI-TOF

Two main companies are providers of MALDI-TOF mass spectrometers: Bruker and BioMéerieux. The spectra produced by the two machines are not completely equivalent, and the softwares and procedures are also different. This makes the two only commercially available mass spectrometers on the market incompatible with each other. As all active partners had access to a Bruker mass spectrometer, the project activities were conducted on this machine. This solution allows to build additional databases of reference spectra to complete the commercial database provided with the mass spectrometer.

2.3.2. Biological resources

Considering the resources of the project, only a fraction of the plant-pathogenic bacterial genera could be assessed over the course of the project, a selection was made. Partner INRAE focussed on *Curtobacterium*, *Pantoea*, *Pectobacterium*, *Pseudomonas* spp. (*syringae* lineage), and *Xylophilus*. Partner NIVIP focussed on *Clavibacter* and *Ralstonia*. Partner JKI focussed on *Erwinia*.

2.3.3. Standardization of culture procedure

The MALDI-TOF mass spectrometer gives information on the proteins produced by the bacteria. Thus, different cell culturing methods can influence the outcomes of the mass spectrometry analysis because media and cell culture conditions influence the metabolic status of cells, including the expression of proteins.
For this reason, the partners decided to standardize the cell culturing protocols in order to ensure that results are repeatable and reproducible (see § 2.4.1).

2.3.4. Sample preparation

For sample preparation, two methods are available. The most streamlined approach consists in transferring the bacterial ooze from a Petri dish directly onto the MALDI-TOF MS plate. Direct

transfer is suitable for most plant pathogenic bacteria, especially Gram-negative bacteria. The bacterial ooze is then overlaid with a saturated solution of a matrix, generally α-cyano-4-hydroxycinnamic acid (HCCA), and the solvent is allowed to evaporate prior to the test. However, for some taxa (*i.e.* Gram-positive bacteria) it is necessary to disrupt the bacterial cell wall to release the proteins before the matrix is applied and the test performed. In this case, formic acid is added to the bacterial ooze on the MALDI-TOF MS plate; after the addition of the matrix and solvent evaporation, the test is performed. This method is the recommended method for the production of reference spectra (Main Spectra Profiles or MSP). Moreover, it can be used for biosecurity reasons (regulated organisms moved between facilities) or for storage. INRAE used the alternative method, the other partners used the direct transfer method for routine analysis. For reference spectra production, the formic-acid or the alternative method were used.

2.3.5. Production of reference spectra

For each genus, the partners determined the most relevant strains for reference spectra production. These strains were chosen either to represent the diversity of the considered taxa, or because they displayed specific features, such as representative of a particular subgroup or displaying specific antibiotic resistance. In the end, the project permitted the production of 117 reference spectra.

2.3.6. Exchange of reference spectra between partners

INRAE and NIVIP tried to exchange a few of reference spectra. This worked well, the new spectra were integrated into their respective in-house databases and could be used along with the commercial database or along with the other in-house databases for analysis purposes.

2.3.7. Validation of MALDI-TOF mass spectrometry for bacterial identification

To validate the use of MALDI-TOF mass spectrometry for the identification of plant pathogenic bacteria, a large panel of strains representing the diversity of the considered taxa, or belonging to other taxa more or less closely related to the considered taxa were analysed. The results for the identification of these known strains were compared with the expected results. This allowed to assess the diagnostic specificity of the tests.

2.3.8. EPPO standard

An EPPO Standard on the use of the MALDI-TOF mass spectrometry for the identification of plant pathogenic bacteria is being written following this project.

2.4. Main results

2.4.1. Standardized procedures for strain cultivation

Table 1: Recommended media, growing temperature and culturing time for bacteria to be analyed with MALDI-TOF mass spectrometry to ensure the best fit with the provided reference spectra.

Bacteria	Growth conditions			
	Medium	MediaDive reference *	Time (days)	Temperature (˚C)
Clavibacter sepedonicus	Yeast Peptone Glucose Agar (YPGA)	MediaDive 1015	2-5	21

Clavibacter michiganensis subsp. *michiganensis*	Nutrient Agar (NA)	MediaDive 1	2-4	28
Curtobacterium spp.	Tryptone Soy Agar (TSA)	MediaDive 535	2	25
Erwinia amylovora	Nutrient Agar (NA) or Lysogeny broth (LB)	MediaDive 1 or MediaDive 381	1-2	28
Pantoea spp.	Tryptone Soy Agar 10%	MediaDive 535	2	25
Pseudomonas spp. (*syringae* lineage)	King's B	In progress	1	25
Pectobacterium spp.	King's B	In progress	1	25
Ralstonia	Yeast Peptone Glucose Agar (YPGA)	MediaDive 1015	2-3	28
Xylophilus spp.	Yeast Peptone Glucose Agar (YPGA)	MediaDive 1015	4	25

*: The MediaDive database is accessible here: https://mediadive.dsmz.de/Data acquisition

2.4.2. Data acquisition

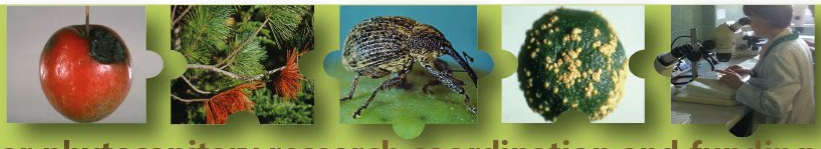
The reference spectra were obtained for 117 strains (*Curtobacterium* spp.: 14, *Clavibacter* spp.: 5, *Erwinia* spp.: 24, *Pantoea* spp.: 12, *Pectobacterium* spp.: 25, *Pseudomonas* spp.: 29, *Ralstonia* spp.: 6, *Xylophilus ampelinus*: 2) (complete list in Annexe 1) with these, a significative number of other strains were analyzed to check the reliability of the technique used as identification method of plant-pathogenic bacteria. At INRAE, 462 known strains were analyzed in total, usually done in duplicate or triplicate.

2.4.3. Validation of MALDI-TOF mass spectrometry for bacterial identification

For each genus, the partners evaluated the ability of the method to correctly identify the strains. It was noted that for a reliable identification, the database should cover the whole known diversity of the considered taxa, including strains representatives of all the different subgroups if such subgroups exist. When the database complies to this criterion, the identification is reliable and correct in all studied cases. However, the limits of a reliable identification varied genera from genera. In some cases, the test can discriminate effectively between species, but for some other genera closely related species are not differentiated. In any case, the method is not able to discriminate at pathovars or subspecies level.

Table 2: Validation of MALDI-TOF MS-based identification at the genus, genus sub-division, species and intra-specific levels. The discrimination limit of the test depends on the considered genus.

	Genus ID	Genomic group ID	Species ID	Subspecies/ pathovar ID
Clavibacter	Yes	na		No
Curtobacterium	Yes	na	*	No
Erwinia	Yes	na	Yes	na



Pantoea	Yes	na	Yes	No
Pectobacterium	Yes	na	No	na
Pseudomonas (*syringae* lineage)	Yes	No	No	No
Ralstonia	Yes	No	Yes	No
Xylophilus	Yes	na	Yes	na

Na: not applicable.

*: The species *Curtobacterium flaccumfaciens* corresponds to a species complex (Goncalvez *et al*., 2019; Osdaghi *et al*., 2022). The discrimination by MALDI-TOF MS of these not-yet described species is currently not possible.

2.4.4. Reference spectra

All 117 reference spectra generated in the framework of this project had been exported from the MALDI-TOF systems they were produced on. They have been gathered, and they have been placed on the public archive of the French ministry for research and available at the following url: https://doi.org/10.57745/7OJNOO. The reference spectra produced in the framework of the project are only compatible with the Bruker system. These data can be used as reference, to complete the commercial database, to help the identification of plant-pathogenic bacteria.

2.5. Conclusions and recommendations to policy makers

The aims of the MALD-ID project were to i) build a database of reference spectra for reliable identification of plant-pathogenic bacteria and to ii) validate the use of MALDI-TOF MS as a diagnostic method in plant-pathology.
During the MALD-ID project, 117 reference spectra were obtained using the Bruker mass spectrometer. The number of reference spectra proved to be sufficient, when used alongside the commercial database provided by Bruker, to reliably identify bacteria from the eight studied taxa (*Clavibacter*, *Curtobacterium*, *Erwinia*, *Pantoea*, *Pectobacterium*, *Pseudomonas* spp. (*syringae* lineage), *Ralstonia* and *Xylophilus*). However, the efficiency of the method to identify strains differs from genera to genera. MALDI-TOF MS is reliable to identify *Pantoea*, *Ralstonia* and *Xylophilus* at species level, but it is not precise enough to discriminate between closely related species for *Curtobacterium*, *Pectobacterium* and *Pseudomonas*. For these three latter genera, the identification at the genus level is correct in any case.

A correct diagnosis in plant-pathology is crucial to implement the correct phytosanitary measures. Often, the relevant taxonomy level for the diagnosis is at the intra-specific level. An example is the *Pantoeae stewartii* subsp. *stewartii*, that is classified as quarantine pest while but *Pantoeae stewartii* subsp. *indologenes* is not. An incorrect diagnostic at this precise taxonomic level can have great consequences for growers and importers. The MALDI-TOF MS allows to correctly identify *Pantoea stewartii* at species level but will not allow to discriminate between strains of the two subspecies.

The MALDI-TOF MS method allows to reliably correctly assign the genus of a given isolate, or even the species in some cases. The method is comparable to the sequencing of 16S rRNA gene, which allows to define the genus, and sometimes the species, of given isolates. The advantage of the MALDI-TOF technique over 16S rRNA gene sequencing is that it is much more rapid, as results can be obtained in about 1 hour. Thus, MALDI-TOF MS can be a powerful tool to be used as a screening method, By allowing to determine the genus of the bacterium, and to help choose the most relevant test for the identification of the pathogen at a

lower level. MALDI-TOF MS can also be used as a screening tool to identify bacterial isolates that are potentially plant-pathogenic.

Therefore, the partners of the MALD-ID project recommend that MALDI-TOF MS is used as a first step in the process of diagnostic. It is recommended to use the reference spectra produced in the framework of this project, or other reference spectra obtained from relevant strains to complete the commercial database provided by the manufacturers.

2.6. Benefits from trans-national cooperation

The project allowed to develop useful data based on strains available in the laboratories of the project partners. This greatly enhanced the diversity of strains, thus allowing a better characterization.

Beyond the data generated in the framework of the project, the collaboration allowed the three partners to enhance their ability to use MALDI-TOF MS for the diagnosis of bacteria. Two training sessions were organized at INRAE, which allowed to train two students. It is hoped that the collaboration started during this project will continue in the future.

References cited in this report

- Gonçalves RM, Balbi-Peña MI, Soman JM, Maringoni AC, Taghouti G, Fischer-Le Saux M, Portier P (2019). Genetic diversity of *Curtobacterium flaccumfaciens* revealed by multilocus sequence analysis. *Eur J Plant Pathol* **154**: 189-202

- Osdaghi E, Taghouti G, Dutrieux C, Taghavi SM, Fazliarab A, Briand M, Le Saux MF, Portier P, Jacques MA (2022). Whole Genome Resources of 17 *Curtobacterium flaccumfaciens* Strains Including Pathotypes of *C. flaccumfaciens* pv. betae, *C. flaccumfaciens* pv. oortii, and *C. flaccumfaciens* pv. poinsettiae. *Mol Plant Microbe Interact* **35**(4): 352-356

- Zaluga J, Heylen K, Van Hoorde K, Hoste B, Van Vaerenbergh J, Maes M, De Vos P (2011). GyrB sequence analysis and MALDI-TOF MS as identification tools for plant pathogenic *Clavibacter*. *Syst Appl Microbiol*. **34**(6): 400-7

3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

None.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

None.

3.4. Other mean of communication

The project activities and results were/will be presented as follows:

- Cécile Dutrieux, Géraldine Taghouti, Audrey Lathus, Claire Darrigo, Perrine Portier. MALDI-TOF technique for identification of plant-pathogenic bacteria: Building of the reference database and evaluation MALD-ID Project. Oral presentation. ICPPB, 14th International Conference on Plant Pathogenic Bacteria. Assisi (Italy) 3-8 July, 2022

- Cécile Dutrieux, Géraldine Taghouti, Audrey Lathus, Léa Vannier, Tatiana Amelon, Perrine Portier. MALDI-TOF technique for identification of plant-pathogenic bacteria: Building of the reference database and evaluation of the method. Poster. IHC, 31st International Horticulture Congress. Angers (France) 14-20 August, 2022

- Cécile Dutrieux, Géraldine Taghouti, Audrey Lathus, Claire Darrigo, Perrine Portier. Mass spectrometry applied to identification of plant-associated bacteria: Matrix Assisted Laser Desorption Ionisation Time Of Flight. Poster. 16° rencontres plantes-bactéries. Aussois (France) 20-24 March, 2023

4. Open Euphresco data

The reference spectra obtained during this project have been deposited on the public repository of the French Ministry for Research 'data.gouv.fr' and are available at the following url: https://doi.org/10.57745/7OJNOO.

Annex 1. List of the strains used for the production of reference spectra

***Pantoea* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
CFBP 2239			*Pantoea agglomerans*		raindeer	1983	USA	
CFBP 4341	Gardan L. 11141	PD 128, NCPPB 3091	*Pantoea agglomerans py gysophilae*	Pathotype	*Gypsophila paniculata*	1978	Netherlands	
CFBP 3615		ICMP 6772, NCPPB 2519	*Pantoea agglomerans pv. miletiae*	Pathotype	*Wisteria floribunda*	1976	Japan	
CFBP 8207	R-27856, PPPPB BD390	LMG 24248	*Pantoea alii*	Type	Onion	2004	South Africa	
CFBP 3612		ICMP 1850, NCPPB 1846	*Pantoea ananatis pv. ananatis*	Type	*Ananas comosus*	1965	Brazil	
CFBP 3171		ICMP 351, NCPPB 800, ATCC 19321	*Pantoea ananatis pv. uredovora*	Pathotype	*Puccinia graminis*	1954	USA	
CFBP 6627		CECT 5260, ATCC 700886	*Pantoea cedenensis*	Type	*Olea europaea*	1998	Spain	
CFBP 3613		ICMP 1591, NCPPB 3004, ATCC 29267, LMG 2657	*Pantoea cypripedii*	Type	*Cypripedium sp.*	<1995	USA	
CFBP 2238			*Pantoea sp*		human	1983	USA	
CFBP 6916		IBSBF 1825	*Pantoea sp.*		*Eucalyptus* hib.	2002	Brazil	
CFBP 8383	ARC 212, Ayome 4, Blitta 8-2		*Pantoea sp.*		*Oryza sativa L.*	2013	Togo	

CFBP 3614		ICMP 77, LMG 2632, NCPPB 2280	*Pantoea stewartii* subsp *indologenes*	Type	*Setaria italica*	<1995	India	

***Pectobacterium* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
CFBP 7370	C331		*Pectobacterium actinidiae*		*Solanum Tuberosum* (Diamant)	2004	Syria	
CFBP 8637	A212-S19-A16	NCPPB 4640	*Pectobacterium aquaticum*	Type	environment/fresh water	2016	France	
CFBP 8168		LMG2417, NCPPB929, PDDCC1522, Dye EC4, Hayward B493	*Pectobacterium aroidearum*	Type	*Zantedeschia aethiopica*	1959	South Africa	
CFBP 1526	Kelman A. SR55	LMG2417, NCPPB929, PDDCC1522, Dye EC4, Hayward B493	*Pectobacterium atrosepticum*	Type	*Solanum tuberosum*	1957	United Kingdom	
CFBP 1539	Vitanza B. Ur7	NCPPB 2795, ATCC 43762, CIP 105193, ICMP 4226, LMG 2466, CFBP 2122	*Pectobacterium betavasculorum*	Type	*Beta vulgaris* cv. Saccharata	1972	USA	
CFBP 6617	Duarte BPBB_212, De Boer S.H. Ecbr_212	IBSBF 1692	*Pectobacterium brasiliense*	Type	*Solanum tuberosum* cv. Elvira	1999	Brazil	

CFBP 3628		NCPPB 3849, ATCC 49481, ICMP 11136, DSM 21821	*Pectobacterium cacticida*	Type	*Carnegiea gigantea*	1944	USA	
CFBP 2046		NCPPB 312, ATCC 15713, CIP 82.83, DSM 30168, HAMBI 1429, ICMP 5702, LMG 2404	*Pectobacterium carotovorum*	Type	*Solanum tuberosum*	1952	Danemark	
CFBP 8629	M022	LMG30744	*Pectobacterium fontis*	Type	fresh water	2013	Malaysia	
CFBP 1878		LMG 5863, CIP 103762, ICMP 11533, NCPPB 3839	*Pectobacterium odoriferum*	Type	*Cichorium intybus*	1978	France	
CFBP 8475	RNS 08-42-1A	LMG29774	*Pectobacterium parmentieri*	Type	*Solanum tuberosum*	2008	France	
CFBP 8630	s0421		*Pectobacterium parvum*	Type	*Solanum tuberosum*	2004	Finland	
CFBP 8603	NIBIO1006	NCPPB 4611	*Pectobacterium polaris*	Type	*Solanum tuberosum*	2010	Norway	
CFBP 8604	SS95	LMG30622	*Pectobacterium punjabense*	Type	*Solanum tuberosum*	2017	Pakistan	
CFBP 6051	De Boer 21	NCPPB 3387, ICMP 9168	*Pectobacterium versatile*	Type	*Solanum tuberosum*	<2001	Netherlands	
CFBP 3304		ICMP 9121, ATCC 43316, CIP 105194, DSM 18074, NCPPB 3701	*Pectobacterium wasabiae*	Type	*Eutrema wasabi*	1985	Japan	

CFBP 8805	A477-S1-J17	LMG 32181	*Pectobacterium quasiaquaticum*	Type	Fresh water	2017	France	
CFBP 5834	Samson R. SL145-CIP009	CIP 009	*Pectobacterium peruviense*		*Solanum tuberosum*	1977	Peru	
CFBP 1357	Lemattre M. 7'3		*Pectobacterium zantedeschiae*		*Zantedeschia sp.*	1964	France	
CFBP 5380			*Pectobacterium sp*		*Solanum tuberosum* cv. Diamant	1997	Netherlands	Pectobacterium "sp 14"
CFBP 6070	De Boer 380	NCPPB 3413, ICMP 9195	*Pectobacterium sp*		*Solanum tuberosum*	<2001	USA	
CFBP 8736	A143-S20-M16		*Pectobacterium sp*		Fresh water	2016	France	
CFBP 797	Burkholder W.H. SR23	ATCC 12286, NCPPB 550	*Pectobacterium sp*		*Nicotiana tabacum*	1951	USA	
CFBP 8739	A644-MFV-A17		*Pectobacterium sp*		Fresh water	2017	France	
CFBP 6588	Gardan L. 11941, GRISP 93.2608, Dufeol D. LNPV-UB 3.24		*Pectobacterium sp*		*Musa* sp.	1993	Martinique (France)	

***Pseudomonas* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
CFBP 7149	Scortini M. 592	ICMP 17001, NCPPB 4273	*Pseudomonas syringae pv. coryli*	Pathotype	*Corylus avellana*	1995	Italy	Pseudomonas cannabina

CFBP 5140		ICMP 13650, MAAF 810036, CFBP 5525	*Pseudomonas syringae pv. Broussonetiae*	Pathotype	*Broussonetia kazinoki* Sieb.X *Broussonetia papyrifa* Vent.	1980	Japan	Pseudmonas amygdali
CFBP 3204	Robbs C.F. ENA378	NCPPB 1873, ICMP 2855, DSM 21109	*Pseudomonas caricapapayae*	Type	*Carica papaya*	1966	Brazil	Pseudomonas caricapapayae
CFBP 2341	Klement Z. L01	NCPPB 1437, CIP 106140, ICMP 2823, LMG 5096	*Pseudomonas cannabina pv cannabina*	Type	*Cannabis sativa*	1957	Hungary	Pseudomonas cannabina
CFBP 8664	LLPA 221	NCPPB 4619, CECT 8095, LMG 26898	*Pseudomonas asturiensis*	Type	*Glycine max*	2001	Spain	Pseudomonas asturiensis
CFBP 2351		NCPPB 2995, ATCC 19322, ICMP 5795, LMG 5075	*Pseudomonas syringae pv. morsprunorum*	Pathotype	*Prunus domestica*	<1984	USA	Pseudomonas avellanae
CFBP 2897	Ogimi C. MR1	ICMP 7118, LMG 5668, ATCC 33544, NCPPB 3143	*Pseudomonas syringae pv. myricae*	Pathotype	*Myrica rubra*	1978	Japan	Pseudmonas amygdali
CFBP 4060	Psallidas P.G. F11, Gardan L. 10963, 11144	NCPPB 3487, BPIC 631, ICMP 9746	*Pseudomonas avellanae*	Type	*Corylus avellana*	1976	Greece	Pseudomonas avellanae
CFBP 4218		ICMP 11894	*Pseudomonas syringae pv. cunninghamiae*	Pathotype	*Cunninghamia lanceolata*	<1997	China	Pseudmonas amygdali
CFBP 2212	Lelliott R.A. T44	NCPPB 1106, ICMP 2844, LMG 5093	*Pseudomonas syringae pv. tomato*	Pathotype	*Lycopersicon esculentum*	1960	United Kingdom	Pseudomonas avellanae
CFBP 2356	Dye D.W. Py5	NCPPB 225, ATCC 19863,	*Pseudomonas syringae pv. Dysoxyli*	Pathotype	*Dysoxylum spectabile*	1949	New Zealand	Pseudomonas syringae

		ICMP 545, LMG 5062;						
CFBP 3228	Neto J.R. IBBF160	ICMP 5809	*Pseudomonas syringae pv. oryzae*		*Oryza sativa*	1983	Japan	"Pseudomonas coronafaciens"
CFBP 2216		NCPPB 600, ICMP 3113, LMG 5060	*Pseudomonas syringae pv. coronafaciens*	Pathotype	*Avena sativa*	1958	United Kingdom	"Pseudomonas coronafaciens"
CFBP 2101		NCPPB 943, ATCC 10857, ICMP 5707, LMG 2162	*Pseudomonas cichorii*	Type	*Cichorium endivia*	1929		Pseudomonas cichorii
CFBP 2107		NCPPB 635, ATCC 13223, CIP 106699, DSM 11124, ICMP 2848, LMG 2352	*Pseudomonas viridiflava*	Type	*Phaseolus sp.*	1927	Switzerland	Pseudomonas viridiflava
CFBP 8878		ICMP7840	*Pseudomonas syringae pv. photiniae*	Pathotype	*Photinia glabra*	1976	Japan	Pseudmonas amygdali
CFBP 2106	Klement Z. H59	NCPPB 1427, ICMP 2835, LMG 5393	*Pseudomonas syringae pv. tabaci*	Pathotype	*Nicotiana tabacum*	1959	Hungary	Pseudmonas amygdali
CFBP 4217		ICMP 9419	*Pseudomonas syringae pv. castaneae*	Pathotype	*Castanea crenata*	1977	Japan	Pseudmonas amygdali
CFBP 3846	Prunier J.P. cc13-6	NCPPB 4290, ICMP 14479	*Pseudomonas syringae pv. avii*	Pathotype	*Prunus avium*	1991	France	Pseudomonas avellanae
CFBP 6109	Takikawa Y. M9501	ICMP 17524	*Pseudomonas syringae pv. cerasicola*	Pathotype	*Prunus X yedoensis*	1995	Japan	Pseudomonas avellanae
CFBP 8305		LMG 28609	*Pseudomonas cerasi*	Type	*Prunus cerasus*	2007	Poland	Pseudomonas cerasi
CFBP 7019	Behrendt U. P538/23	CIP 108180, DSM 14939,	*Pseudomonas congelans*	Type	*Graminaea*	1994	Germany	Pseudomonas congelans

		LMG 21466 ICMP 19117						
CFBP 4909	takikawa Y. KW11, Gardan L. 11325	ICMP 9617, NCPPB 3739	*Pseudomonas syringae pv. actinidiae*	Pathotype	*Actinidia deliciosa*	1984	Japan	Pseudomonas avellanae
CFBP 1670		ATCC 13522, NCPPB 639, ICMP 4352, LMG 2209	*Pseudomonas savastanoi pv. savastanoi*	Pathotype	*Olea europaea*	<1976	Yugoslavia	Pseudmonas amygdali
CFBP 1694	Bradbury J.F. B5063	NCPPB 2488, ICMP 4091, LMG 5090	*Pseudomonas syringae pv. tagetis*	Pathotype	*Tagetes erecta*	1972	Zimbabwe	Pseudomonas caricapapayae
CFBP 2215		NCPPB 1879, ICMP 529, LMG 5381	*Pseudomonas syringae pv.delphinii*	Pathotype	*Delphinium sp.*	1957	New Zealand	Pseudomonas avellanae
CFBP 4220		ICMP 9756, ATCC 49212, NCPPB 3618	*Pseudomonas syringae pv. rhaphiolepidis*	Pathotype	*Rhaphiolepis umbellata*	1980	Japan	Pseudmonas amygdali
CFBP 5524	Ozaki 8605	MAFF 211266	*Pseudomonas syringae pv. spinaceae*	Pathotype		<2001	Japan	Pseudomonas avellanae
CFBP 8039	LSV 38.26		*Pseudomonas syringae pv. actinidifoliorum*	Pathotype	*A. deliciosa*	2011	France	Pseudomonas avellanae

***Curtobacterium* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
CFBP 3401		LMG 7238, PDDCC 4735	*Curtobacterium flaccumfaciens pv. betae*		*Beta vulgaris*	<1994	United Kingdom	Genomic Group III (Goncalvez *et al.*, 2019)
CFBP 3509		LMG 3596, ICMP 2594,	*Curtobacterium flaccumfaciens pv. betae*	Pathotype	*Beta vulgaris*	1955	United Kingdom	Genomic Group III (Goncalvez *et al.*, 2019)

		LMG 3596, NCPPB 374						
CFBP 3406		LMG 7243	*Curtobacterium flaccumfaciens pv. flaccumfaciens*		*Phaseolus vulgaris*	<1994		Genomic Group II (Goncalvez *et al.*, 2019)
CFBP 3418	Klement Z. E1429	NCPPB 1446, ICMP 2584, LMG 3645, CFBP 3456	*Curtobacterium flaccumfaciens pv. flaccumfaciens*	Pathotype	*Phaseolus vulgaris*	1957	Hungary	Genomic Group II (Goncalvez *et al.*, 2019)
CFBP 3419		NCPPB 1597	*Curtobacterium flaccumfaciens pv. flaccumfaciens*		*Phaseolus vulgaris*	1954		Genomic Group III (Goncalvez *et al.*, 2019)
CFBP 3423		NCPPB 2344, ATCC 23827	*Curtobacterium flaccumfaciens pv. flaccumfaciens*		*Phaseolus vulgaris*	1957	USA	Genomic Group III (Goncalvez *et al.*, 2019)
CFBP 8391	CANO-3191, Strain 673		*Curtobacterium flaccumfaciens pv. flaccumfaciens*		*Brassica Napus L.*	2013	Brazil	Genomic Group I (Goncalvez *et al.*, 2019)
CFBP 8879		ICMP 2608	*Curtobacterium flaccumfaciens pv. illicis*	Pathotype	*Ilex opaca Ait.*	1960	USA	Genomic Group III (Goncalvez *et al.*, 2019)
CFBP 1384	Maas Geesteranus H.P. B360	NCPPB 2113, ATCC 25283, ICMP 2632, LMG 3702	*Curtobacterium flaccumfaciens pv. oortii*	Pathotype	*Tulipa gesneriana*	1967	Netherlands	Genomic Group III (Goncalvez *et al.*, 2019)
CFBP 3399		PD 915	*Curtobacterium flaccumfaciens pv. oortii*		*Tulipa sp.*	1987	Netherlands	Genomic Group III (Goncalvez *et al.*, 2019)
CFBP 3400		PD 1751	*Curtobacterium flaccumfaciens pv. oortii*		*Zantedeschia aethiopica*	1990	Netherlands	At the species limit
CFBP 3415		LMG 7321	*Curtobacterium flaccumfaciens pv. poinsettiae*		*Euphorbia pulcherrima*	<1994	USA	Genomic Group I (Goncalvez *et al.*, 2019)

CFBP 2403	Starr M.P. Cp2	ICMP 2566, ATCC 9682, NCPPB 854	*Curtobacterium flaccumfaciens pv. poinsettiae*	Pathotype	*Euphorbia pulcherrima*	<1994	USA	Genomic Group I (Goncalvez *et al.*, 2019)
CFBP 3438		NCPPB 844	*Curtobacterium flaccumfaciens pv. poinsettiae*		*Euphorbia pulcherrima*	1958	USA	Genomic Group I (Goncalvez *et al.*, 2019)

***Xylophilus* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
CFBP 1192	Panagopoulos C.G. 48	NCPPB 2217, ICPB Xa138, ICMP 4298, ATCC 33914, LMG 5949, DSM 3584, CFBP 3674	*Xylophilus ampelinus*	Type	*Vitis vinifera* cv. Sultana	1966	Greece	
CFBP 2098	Ridé M. P6131	LMG 514	*Xylophilus ampelinus*		*Vitis vinifera* cv. Grenache	1979	France	

***Clavibacter* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
PD 5069	RICP 12/5/98		*Clavibacter insidiosus*		*Medicago sativa*	-	Czech Republic	
PD 7217			*Clavibacter michiganensis* subsp. *michiganensis*		*Solanum lycopersicum*	2016	Netherlands	
PD 174			*Clavibacter nebraskensis*		*Zea mays*	1979	USA	
PD 330		LMG 6385	*Clavibacter sepedonicus*		*Solanum tuberosum*	1982	Norway	

PD 336	Vidaver 78181	ATCC 33566; ICMP 7221, PDDCC 7221; LMG 7294	*Clavibacter tesselarius*	Type	*Triticum aestivum*	1982		

***Ralstonia* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
PD 7243	R292	NCPPB 4012	*Ralstonia pseudosolanacearum*		*Morus*	≤1997	China	phylo I
PD 1940	Baigent PU21; Hayward B509; UQRS 461	LMG 9673; ICMP 769; NCPPB 1029	*Ralstonia pseudosolanacearum*	Type	*Pelargonium capitatum*	1961	Reunion	phylo III
PD 3272	SMT 44, CIP312, R578	NCPPB 3985; CFBP 4612	*Ralstonia solanacearum*		*Solanum melongena*	1989	Peru	phylo II
PD 7244	ICMP 10002; T394X	NCPPB 3727	*Ralstonia syzygii* subsp. *celebesensis*		*Musa*	≤1990	South Sulawesi	phylo IV
PD 2889	B8719a	NCPPB 3219	*Ralstonia syzygii* subsp. *indonesiensis*		*Syzygium aromaticum*	1980	Indonesia	phylo IV
PD 7246	S442 (R002)	NCPPB 3445	*Ralstonia syzygii* subsp. *syzygii*		*Syzygium aromaticum*	≤1985	Java, Indonesia	phylotype IV

***Erwinia* spp.**

Strain name	Other names	Name in collections	Taxonomy	Type strain?	Isolation host	Isolation year	Country of origin	Comment about taxonomy
CFBP 1232		NCPPB 683, ATCC 15580, CCM 1114	*Erwinia amylovora*	Type	*Pyrus communis*	1959	United Kingdom	
CFBP 1430			*Erwinia amylovora*		*Crataegus oxyacantha*	1972	France	

IL6			*Erwinia amylovora*					rubus strain
NCPPB 660			*Erwinia billingiae*		*Crataegus oxycantha*	1959	United Kingdom	
NCPPB 661		DSM 17872, LMG 2613	*Erwinia billingiae*	Type	*Pyrus communis*	1959	United Kingdom	first described as Erwinia gerundensis
EM 486			*Duffyella gerundensis*					first described as Erwinia gerundensis
EM 595		CFBP 8471, LMG 28990	*Duffyella gerundensis*	Type	*Pyrus communis* cv. Winter Nellis, Leaf	1994	Spain	
CFBP 2503		NCPPB 2851, ATCC 29573, ICMP 5705, LMG 2708	*Erwinia mallotivora*	Type	*Mallotus japonicus*	1975	Japan	
CFBP 5189		NCPPB 4294, DSM 16540	*Erwinia papayae*	Type	*Carica papaya* cv. local	1995	Martinique (France)	strains of E. persicina and E. rhapontici are sometimes intermixed in databases
CFBP 3622		ATCC 35998, CIP 105199, ICMP 12532, LMG 11254, NCPPB 3774	*Erwinia persicina*	Type	*Lycopersicon esculentum*		Japan	
CFBP 5888			*Erwinia piriflorinigrans*	Type	*Pyrus communis* var. Ercolini			
IVIA 2055			*Erwinia piriflorinigrans*					
CFBP 3627		NCPPB 3555, ICMP 8426	*Erwinia psidii*	Type	*Psidium guajava*		Brazil	
DSZM 12393	Ep8/95		*Erwinia pyrifoliae*		*Pyrus pyrifolia*		Republic of Korea	

Ejp546			*Erwinia pyrifoliae*					
DSM 12162	Ep 1/96		*Erwinia pyrifoliae*		*Pyrus pyrifolia*		Republic of Korea	strains of E. persicina and E. rhapontici are sometimes intermixed in databases
CFBP 3163	CFBP 3618		*Erwinia rhapontici*	Type	*Rheum rhabarbarum*		United Kingdom	
Et1/99		CFBP 7177, DSM 17950, NCPPB 4357, CIP 109463	*Erwinia tasmaniensis*	Type	*Malus sp.*	1999	Australia	
Et2/99		DSM 17949	*Erwinia tasmaniensis*		pear flowers	1999	Australia	
NCPPB 4475		LMG 25843, NCPPB 4475, YPPS 951	*Erwinia uzenensis*	Type	*Pyrus communis* L cv. La France (European Pear)		Japan	

Supplementary strains for comparison of wt strains vs Sm mutants

CFBP 1430Sm			*Erwinia amylovora*					
Ejp546Sm			*Erwinia pyrifoliae*					
Ep 1/96 Sm			*Erwinia pyrifoliae*					
Et1/99 Sm			*Erwinia tasmaniensis*					