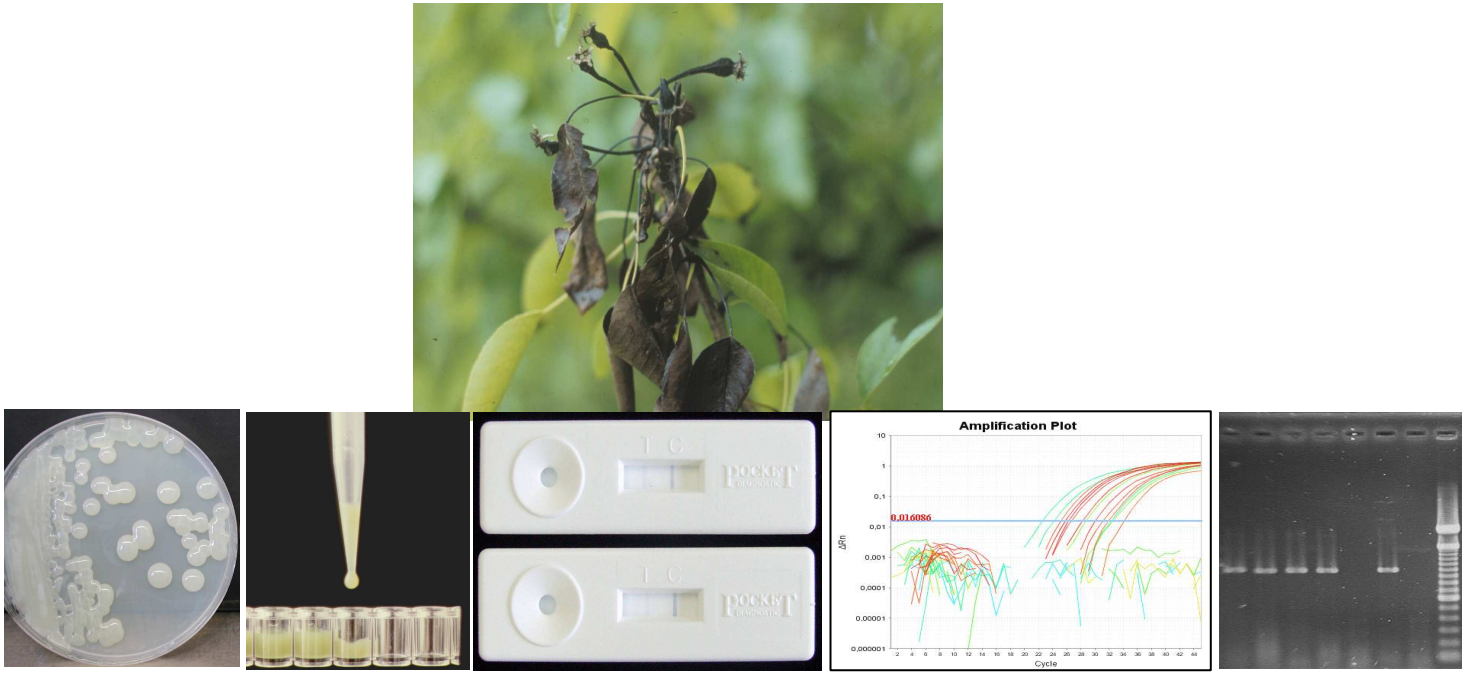
ROUTINE DIAGNOSIS OF *Erwinia amylovora* AND VALIDATION. DETECTION IN ASYMPTOMATIC PLANTS



MARÍA M. LÓPEZ

Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Valencia

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Erwinia species from pome fruit trees

Pathogenic:

- ***Erwinia amylovora***
- ***Erwinia pyrifoliae***
- ***Erwinia* sp. from Japan**
- ***Erwinia piriflorinigrans***
- ***Erwinia uzenensis***

Non pathogenic:

Erwinia billingiae

Erwinia tasmaniensis

- **Some *Erwinia* sp. from Japan have been classified as *Erwinia pyrifoliae.***

- All these *Erwinia* species are phylogenetically related.

- Restricted host range for pathogenic strains .

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Erwinia amylovora

- **Described by Burrill in 1883.**
- **Present in N. America, Europe, Middle Est, N. Africa.**
- **Quarantine organism in the EU and the EPPO region.**
- **Responsible of fire blight disease.**
- **Large geographical distribution and economic importance.**
- **Rosaceous hosts:**
  fruit trees (pear, apple, quince, loquat),
  ornamental and wild plants
  (cotoneaster, pyracantha,
  stranvaesia, hawthorn, sorbus)

Erwinia pyrifoliae

- **Described by Kim *et al*., 1999.**
- **Reported in Korea and Japan (*Erwinia* sp.).**
- **Affects *Pyrus pyrifolia* and *Pyrus communis.***
- **Symptoms similar to those of *E. amylovora.***
- **Phenotypic characteristics similar to *E. amylovora.***
- **DNA-DNA hybridization assays demonstrated that it was a new species.**

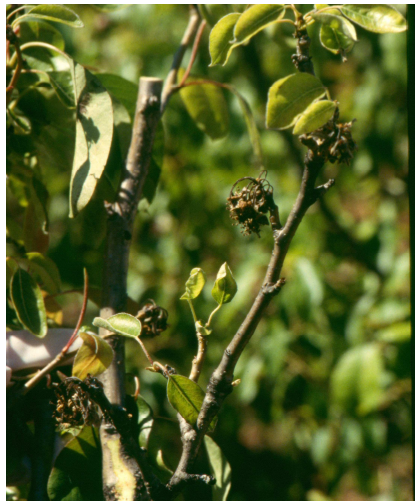
Foto: Shrestha, 2003

Erwinia piriflorinigrans

- **Roselló y col., 2006 - López y col., 2011.**
- **Only found in Valencia, Spain, up to now.**
- **Symptoms similar to those of *E. amylovora* but only on pear blossoms.**
- **Analyses of 16S rRNA sequences showed high similarity to *E. amylovora*, *E. pyrifoliae*, *E. tasmaniensis* and *E. billingiae***

DNA-DNA hybridization assays demonstrated that it was a new species.

Symptoms observed on pear



- 30 ha of Ercolini (Coscia) and Tendral were affected in 1999, 2000 and 2001 in Valencia.

- Necrotic flowers, no shoots affected.

- Other fire blight hosts were not affected in the same area.

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Identification of an *Erwinia* sp. different from *Erwinia amylovora* and responsible for necrosis on pear blossoms

M. Roselló, J. Peñalver, P. Llop, M.T. Gorris, R. Chartier, F. García, C. Montón, M. Cambra, and M.M. López

Abstract: Necrotic pear blossoms (NPB) were found in several pear orchards of 'Ercolini' ('Coscia') and 'Tendral' in Turís, Valencia (Spain), in April 1999, during a routine survey, in an area free of *Erwinia amylovora*. The symptoms resembled those of fire blight [*E. amylovora*] but affected only blossoms and did not progress to other parts of the tree. *Erwinia*-like colonies were isolated from the necrotic blossoms that year and the following 2 years, and the morphology of the colonies on CCT medium of Ishimaru and Klos, King's B medium, and sucrose nutrient agar was similar to that of *Erwinia amylovora*. The isolates were identified as an *Erwinia* sp. by their microbiological characteristics and showed API 20E, API 20NE, API ZYM, API 50CH patterns and fatty acid profiles similar, but not identical, to those of *E. amylovora* and *Erwinia pyrifoliae*. The isolates reacted as *E. amylovora* in immunofluorescence with several antisera and one monoclonal antibody (MAb) employed for *E. amylovora* detection, but did not react in the enzyme-linked immunosorbent assay against specific *E. amylovora* monoclonal antibodies. Polymerase chain reaction with primers from 23S rDNA sequences of *E. amylovora* were positive, but no signal was obtained with primers from plasmid pEa29 or from chromosomal DNA sequences of *E. amylovora*. The isolates were able to elicit the hypersensitive reaction on tobacco and tomato leaves and to induce necrosis in pear flowers, but were unable to develop typical fire blight symptoms in other organs of pear trees, and on various host plants of *E. amylovora*. The isolated *Erwinia* sp. strains are pathogenic and different from *E. amylovora* and other described bacterial species affecting pear trees.

Key words: phenotypic characterization, *Erwinia amylovora*, *Erwinia* sp., fatty acid analysis, pathogenicity, polymerase chain reaction, fire blight, *Pyrus communis*.

Résumé : En avril 1999, au cours d'une inspection routinière dans une zone exempte d'*Erwinia amylovora*, des bouquets floraux nécrosés ont été observés dans des parcelles de poirier 'Ercolini' ('Coscia') et 'Tendral' à Turís, Valencia (Espagne). Les symptômes ressemblaient à ceux du feu bactérien [*E. amylovora*], mais ils affectaient seulement les fleurs et n'avançaient pas dans d'autres parties de l'arbre. Des colonies bactériennes de type *Erwinia* ont été isolées des fleurs nécrosées cette année et les deux années suivantes. La morphologie des colonies était similaire à celle de l'*Erwinia amylovora* sur milieu CCT d'Ishimaru et Klos, sur milieu King's B et sur agar nutritif enrichi en saccharose. Les isolats ont été identifiés comme étant une espèce d'*Erwinia* d'après leurs caractéristiques microbiologiques. Les souches ont montré des résultats en API 20E, API 20NE, API ZYM et API 50CH et des profils en acides gras proches de ceux de l'*E. amylovora* et de l'*Erwinia pyrifoliae*, mais non identiques. Elles réagirent comme l'*E. amylovora* en immunofluorescence avec plusieurs antisérums et un anticorps monoclonal employé pour la détection de l'*E. amylovora*, mais elles ne réagirent pas en essais immunoenzymatiques avec des anticorps monoclonaux spécifiques à l'*E. amylovora*. La réaction de polymérisation en chaîne avec une paire d'amorces des séquences d'ADNr 23S d'*E. amylovora* était positive, mais il n'y avait pas d'amplification avec des amorces du plasmide pEa29 ou des séquences chromosomiques de l'ADN d'*E. amylovora*. Les isolats ont provoqué la réaction d'hypersensibilité sur des feuilles de tabac et de tomate

Canadian Journal of Plant Pathology 28: 30-41 (2006)

Polyphasic approach for taxonomic classification of the strains of *Erwinia* sp.

- Phenotypic characterization
- Fatty acid analysis
- PCR amplification
- Plasmid profile
- 16S rDNA sequencing
- DNA-DNA hybridization

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Erwinia piriflorinigrans sp. nov.

Erwinia from pear (pirus, Latin name for pear) flower (flos, Latin name for flower) blackened (nigrans from the Latin verb nigrare)

López et al. IJSEM, 2011.

Pathogenicity of *E. piriflorinigrans*

- HR on tobacco +
- Pear blossoms +
- Pear shoots -
- Pear fruitlets -
- Apple, quince, loquat, pyracantha -

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Erwinia uzenensis

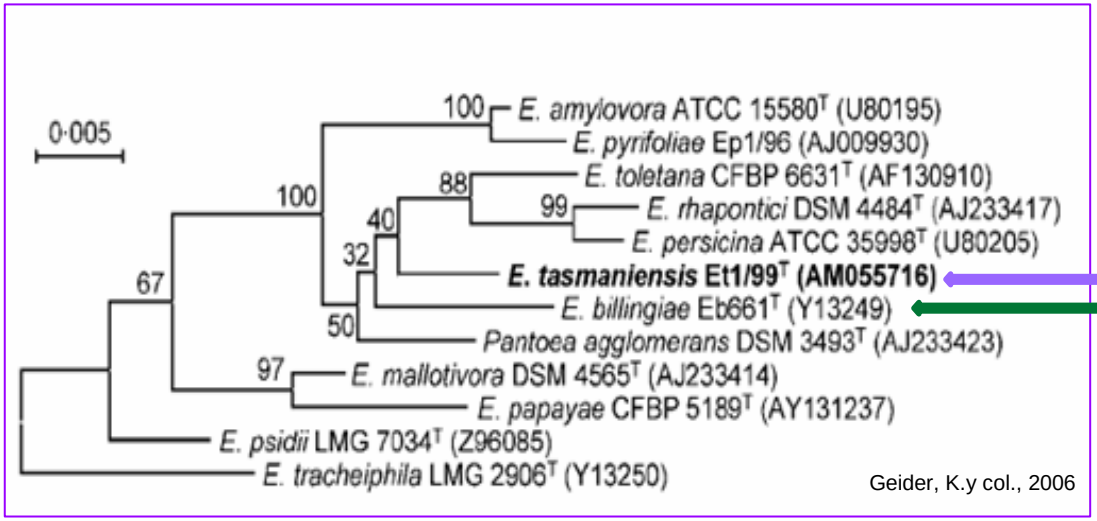
- **Mitsuura *et al*., (2012).**
- **Reported only in Yamagata prefecture in Japan.**
- **Symptoms similar to those of *E. amylovora* on pear cv. La France.**
- **Similar phenotypic characteristics to other Erwinia species.**

DNA-DNA hybridization assays demonstrated that it was a new *Erwinia* species.

Non pathogenic *Erwinia*

- ***Erwinia tasmaniensis***
  - Described by Geider *et al*., 2006.
  - Reported in Australia.
  - Isolated from pear and apple .
  - Epiphytic species related to pathogenic and non pathogenic *Erwinia*
  - HR positive in tobacco

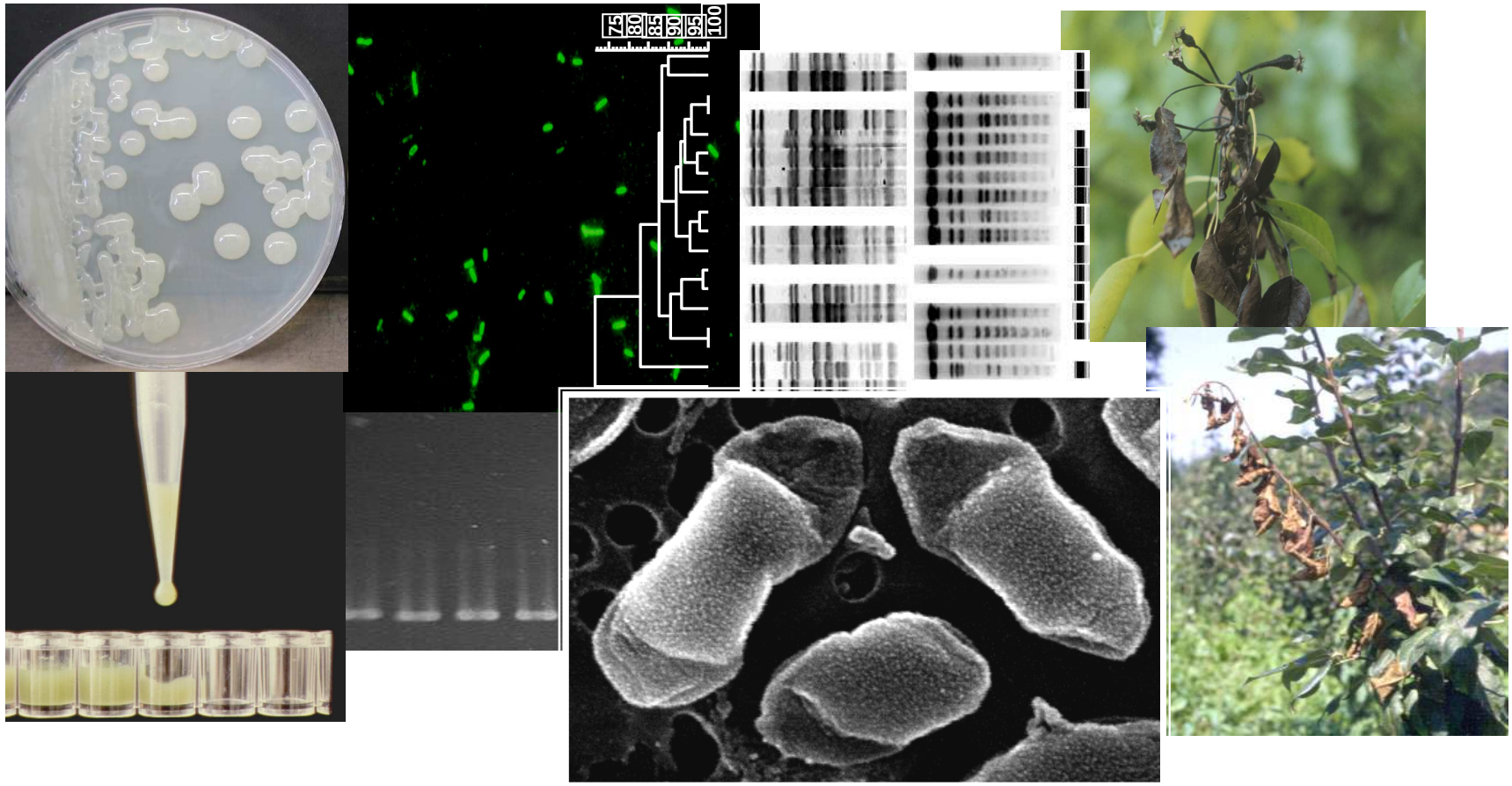
- ***Erwinia billingiae***
  - Described by Mergaert *et al* ., 1999.
  - Reported in Europe.
  - Isolated from pear.



All this Erwinia species share:

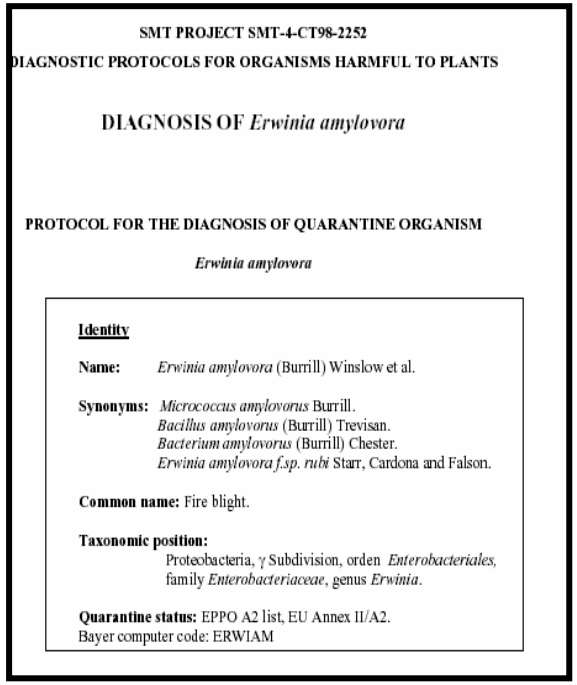
- **Plant habitat: rosaceous hosts**
- **Epiphytic and endophytic life**
- **Some can cause symptoms similar to those of fire blight disease.**
- **Not well known geographical distribution and economic importance.**
- **Many common genes**
- **NEED OF ACCURATE DETECTION AND IDENTIFICATION METHODS !!!**

Erwinia amylovora



Diagnosis of *Erwinia amylovora*

EPPO Bulletin, 34: 159-171
2004



SMT PROJECT SMT-4-CT98-2252

DIAGNOSTIC PROTOCOLS FOR ORGANISMS HARMFUL TO PLANTS

DIAGNOSIS OF *Erwinia amylovora*

PROTOCOL FOR THE DIAGNOSIS OF QUARANTINE ORGANISM

Erwinia amylovora

Identity

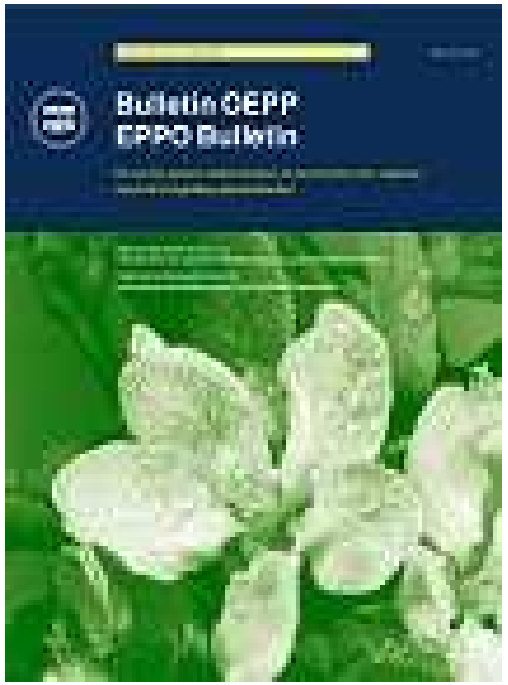
Name: *Erwinia amylovora* (Burrill) Winslow et al.

Synonyms: *Micrococcus amylovorus* Burrill.
Bacillus amylovorus (Burrill) Trevisan.
Bacterium amylovorus (Burrill) Chester.
Erwinia amylovora f.sp. rubi Starr, Cardona and Falson.

Common name: Fire blight.

Taxonomic position:
Proteobacteria, γ Subdivision, orden *Enterobacteriales*, family *Enterobacteriaceae*, genus *Erwinia*.

Quarantine status: EPPO A2 list, EU Annex II/A2.
Bayer computer code: ERWIAM



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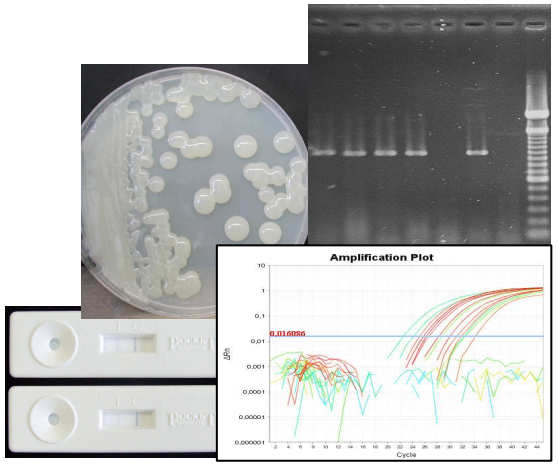
2009
EUPHRESCO RING TEST FOR *E. amylovora*

Published diagnostic tests for *E. amylovora* were evaluated and compared to the techniques of the EPPO protocol (EPPO, 2004) in the European project ERWINDECT (EUPHRESCO, EUropean PHytosanitary RESearch COordination).

- 5 participants: (AGES, AT; NIB, SL; LNPV,F; ACW, CH; IVIA, ES).

- 10 samples, healthy and from 1 to 10^6 cfu/ml

- 4 techniques: isolation, serological kits, conventional PCR, real-time PCR.



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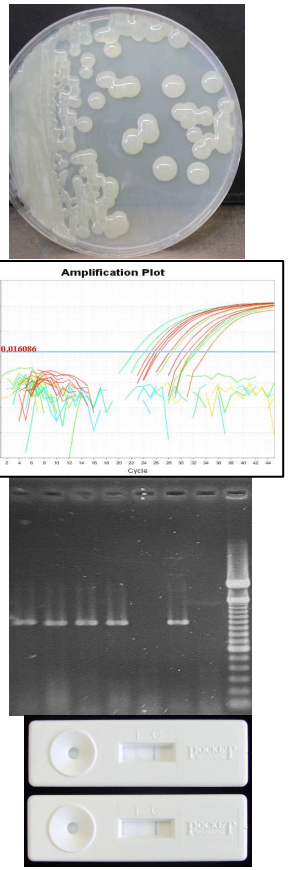
RING TEST RESULTS (2009)

DETECTION TECHNIQUES		AC*
Isolation	King B Medium	0,96
RT PCR (Pirc *et al*, 2009)	DNA extraction – Taylor	0,73
PCR (Llop *et al*, 2000)	DNA extraction – Isopropanol	0,67
PCR (Llop *et al*, 2000)	DNA extraction – Taylor	0,76
PCR (Obradovic, 2007) modified by Gottsberger.	DNA extraction – Taylor	0,76
PCR (Stoger *et al*, 2006)	DNA extraction – Redextract	0,76
PCR (Taylor *et al*, 2001)	DNA extraction – Taylor	0,75
Pocket Diagnostic	with kit buffer	0,64
Agristrip	with kit buffer	0,66



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*AC=ACCURACY: true positives + true negatives / total number of samples

2010

- EPPO, revised version of the EPPO *Erwinia amylovora* protocol.

- International Plant Protection Convention DIAGNOSTIC PROTOCOL FOR *Erwinia amylovora:* M. M. López, R. Taylor, R. Roberts.

- Objective:

  - To design an EPPO and IPPC protocols for diagnosis of *E. amylovora* in symptomatic and asymptomatic plant material.

  - Review of available detection and characterisation methods.
  - Pilot studies: comparative evaluation of sensitivity and specificity.
  - Production of a diagnostic protocol and reference materials.
  - Standardization of methods, production of kits for ring test.

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E. amylovora RING TEST (2010)

Laboratory	City, Country	Scientist
AGES	Vienna, AUSTRIA	H. Reisenzein
CDR	Salamanca, SPAIN	J. L. Palomo
IVIA	Valencia, SPAIN	M. M. López
LRBV	Logroño, SPAIN	M. Marín
LNPV	Angers, FRANCE	F. Poliakoff
FS	Meknès, MOROCCO	M. Mohieddine
INRA	Meknès, MOROCCO	E. H. Achbani
NAK	Roelofarendsveen, THE NETHERLANDS	E. T. M. Meekes
PD	Wageningen, THE NETHERLANDS	M. Bergsma-Vlami
MAF	Auckland, NEW ZEALAND	R. Taylor
RCPQ	Moscow, RUSSIA	N. Drenova
NIB	Ljubljana, SLOVENIA	T. Dreo
UBFA	Belgrade – Zemun, SERBIA	A. Obradovic
OSU	Corvallis, OR, EEUU	V. Stockwell

E. amylovora RING TEST

- 13 samples:

- 7 spiked samples from 10^6 to 1 cfu/ml
- 6 blind samples (negative)

Asymptomatic samples simulated by spiking healthy host samples with low levels of *E. amylovora*

- Techniques and protocols:

- Isolation (3 media)
- Enrichment-isolation (2 media)
- Enrichment DASI-ELISA (2 media)
- Agristrip
- Pocket Diagnostic
- PCR (4 protocols, 3 DNA extraction methods)
- Real-time PCR (1 protocol, 3 DNA extraction methods)
- LAMP

- Sent to each laboratory:

- Protocols, reference strain, plant material, serological kits, ELISA-kit, DNA extraction kits, primers, buffers, polymerases

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Analysis of diagnostic tests

- SENSITIVITY= true positives/(true positives+ false negatives)=

total real positives

- SPECIFICITY= true negatives/(true negatives+false positives)=

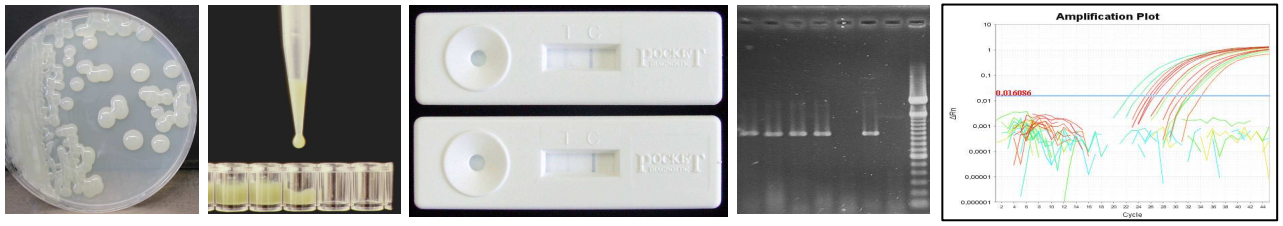
total real negatives

- POSITIVE PREDICTIVE VALUE= true positives/(true positives+false positives)

- NEGATIVE PREDICTIVE VALUE= true negatives/(true negatives+false negatives)

- HIT RATE (ACCURACY) = (true positives+true negatives)/ total samples

http://www.olmosantonio.com



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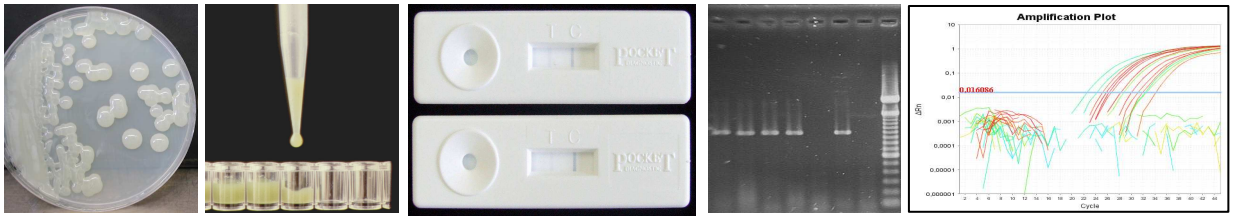

- The reliability of the diagnosis across all laboratories decreases with bacterial concentration.

- The order of accuracy was: enrichment-isolation> isolation > real-time PCR> enrichment DASI-ELISA> PCR (Obradovic *et al.*, 2007)> PCR (Taylor *et al.*, 2001)> PCR (Stöger *et al.*, 2006)= PCR >LAMP > Agristrip = PocketDiagnostic.

- *E. amylovora* detection (asymptomatic) requires an integrated approach that should include the enrichment before any technique and isolation for confirmation.

- The revised EPPO and IPPC protocols have been submitted for final evaluation in 2012.

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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 7/20(1)**REV**

For final adoption

Diagnostics
Diagnostic

Erwinia amylovora[1]

Specific scope
This standard describes a diagnostic protocol for *Erwinia amylovora.*

Specific approval and amendment
This Standard was developed under the EU DIAGPRO Project (SMT 4-CT98-2252) and EUPHRESCO Pilot project (ERWINDECT) by partnership of contractor laboratories. Test performance studies were performed with different laboratories in 2002, 2009, and 2010.
Approved as an EPPO Standard in 2003-09.
Revised in 20xx-09

Introduction
Erwinia amylovora is the causal agent of fire blight in most species of the subfamily *Maloideae* of the family *Rosaceae*. The most economically important hosts are *Pyrus* spp., *Malus* spp., *Cydonia* spp., *Eriobotrya japonica*, *Cotoneaster* spp., *Crataegus* spp., *Pyracantha* spp. and *Sorbus* spp. Other hosts include *Chaenomeles*, *Mespilus* and *Photinia.* A *forma specialis* was described from *Rubus* spp. (Starr *et al.*, 1951; Bradbury, 1986). An exhaustive list of affected plants, including those susceptible only after inoculation, was reported by van der Zwet & Keil (1979). It includes more than 180 species from 39 genera of the *Rosaceae*. *E. amylovora* was the first bacterium described as a causal agent of a plant disease by Burrill (1883). It was reported in North America and was later detected in New Zealand in 1920. In Europe, fire blight was reported in 1957 in the United Kingdom and has since been identified in most areas where susceptible hosts are cultivated. *E. amylovora* is now present in more than forty countries (van der Zwet, 2002; CABI/EPPO, 2007), but it has not been recorded either in South America, Asia or in sub Saharan African countries. It has been recorded in some North African countries and only once in Australia (Bonn & van der Zwet, 2000). It represents a threat to the pome fruit industry of all the countries. Details on geographical distribution can be found in the EPPO Plant Quarantine data Retrieval system (PQR, 2012).
Fire blight is probably the most serious disease affecting pear or apple cultivars in many countries. Although the life cycle of the bacterium is still not fully understood, it is known that it can survive as endophyte or epiphyte for variable periods of time depending of environmental

[1] **Use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.**

INTERNATIONAL STANDARDS FOR

PHYTOSANITARY MEASURES

Annex to ISPM No. 27 (DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS)

Erwinia amylovora

Date of document: July 2010 / Revised version May 2012

Consultation at technical level: The first draft of this protocol was written by M.M. López, M.T. Gorris, P. Llop, V. Donat, J. Peñalver, M. Cambra, R. Roberts and R. Taylor. Some techniques described were ring tested in the DIAGPRO project financed by the EU. The participants in the 2003 ring test for evaluating the *E. amylovora* detection techniques were laboratories from The Netherlands (1), Austria (1), Norway (1), Spain (3), UK (1), Portugal (1), France (1), and Belgium (1). The results were published in Acta Horticulturae (López *et al*., 2006) and form the basis of the EPPO protocol (Anonymous, 2004), revised in 2012.

This revised version was written by the same authors with the cooperation of I. Navarro, A. Arilla and B. Álvarez. The techniques described were ring tested first in 2009 in the context of an EUPHRESCO project by five EU participants from Austria, Spain, Slovenia, France and Switzerland. The final report is available (www.euphresco.org/downloadFile.cfm?id=662). The new and more efficient techniques and protocols were then ring tested in 2010 by 14 laboratories from Austria (1), Spain (3), France (1), Morocco (2), The Netherlands (2), New Zealand (1), Russia (1), Slovenia (1), Serbia (1) and the USA (1) using healthy samples and healthy samples co-mixed with inoculum levels from 1 to 10^6 cfu/ml. The protocol for the ring test was agreed by the participants beforehand and the summary of the results is also available (López et al.,2010).

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SCHEME FOR SYMPTOMATIC FIRE BLIGHT SAMPLES

Plants with symptoms

RAPID SCREENING TESTS,
Enrichment DASI-ELISA, Agristrip, Pocket Diagnostic
PCR, real-time PCR

ISOLATION, ENRICHMENT-ISOLATION

Negative

Positive

Colonies with typical morphology

Yes

No

IDENTIFICATION TESTS

Yes

No

E. amylovora
not detected

E. amylovora
detected

Confirm pathogenicity

E. amylovora confirmed

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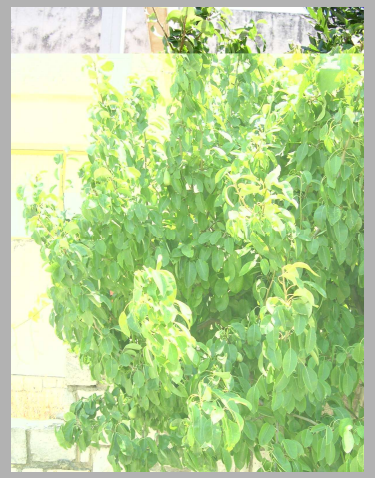
SCHEME FOR ASYMPTOMATIC FIRE BLIGHT SAMPLES

Asymptomatic sample

Pathogen extraction and enrichment

SCREENING TESTS
Isolation, enrichment-isolation , enrichment DASI-ELISA, enrichment-PCR, enrichment-real-time PCR



At least one test positive

Tests all negative

Enrichment isolation

Colonies with typical morphology

No

E. amylovora not detected

Yes

IDENTIFICATION TESTS

No

Yes

E. amylovora detected

Confirm pathogenicity

E. amylovora confirmed

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Survey on the procedures for the analysis of asymptomatic plants for detection and identification of *E. amylovora*

- **Panel on Diagnostics in Bacteriology (Sofia, 2011): a survey organized on the sampling and testing procedures for asymptomatic material.**

- **A questionnaire was developed by IVIA, ES and posted online on April 2012.**

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Sampling procedure for testing PM 7/20 (2004)

- 6 laboratories (Belgium, the Netherlands, Portugal, Slovakia, Spain and United Kingdom) perform both single plant analyses and composite samples.

- 4 laboratories (Austria, Bulgaria, the Czech Republic and Switzerland) perform single plant analyses only.

- 3 laboratories (France, Lithuania and Slovenia) perform composite sample analysis only.

Nb of bulked units	3	4	5	10	30	100
Country of the respondent	Belgium	Portugal	Spain	France	United Kingdom	Lithuania, The Netherlands, Slovakia, Slovenia

Protocol used for testing

- 4 laboratories follow PM 7/20 (2004) for testing.
- 9 also perform other tests.

Other tests or procedures used

Austria, Belgium, France, The Netherlands, Portugal, Slovenia, Switzerland

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Techniques used for testing asymptomatic samples (I)

Technique	Nº labs	Reference
Isolation	8	*Austria, France, the Netherlands, Portugal, Spain, United Kingdom, Bulgaria, Czech Republic*
Enrichment isolation	9	*Austria, Portugal, Slovenia, Spain, Czech Republic, Belgium, Lithuania, The Nethelands, Slovakia*
Immunofluorescence	7	*Czech Republic, Frande, United Kingdom, Bulgaria, Lithuania, The Netherlands, Slovakia*
Enrichment-DASI-ELISA	1	*Spain*

Techniques used for testing asymptomatic samples (II)

Technique	Nº labs	Reference
PCR	7	*Czech Republic, Spain, Austria, Belgium, The Netherlands, Portugal, Slovakia*
Enrichment-PCR	4	*Portugal, Lithuania, Slovakia, Spain*
Real-time PCR	6	*Austria, Lithuania, Slovakia, Spain*
Bioassay	7 +(1[2])	*Austria, Belgium, Portugal, Bulgaria, Czech Republic, Lithuania, The Netherlands, Slovakia*
Other	3	*Belgium, Czech Republic, The Netherlands*

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ASYMPTOMATIC PLANTS :
Nb of positive *E. amylovora* tests/ Nb of tests performed per year

Country of the respondent	2007	2008	2009	2010	2011
Austria	2095/3345	2369/4495	2567/3918	2145/5839	1220/4936
Belgium	0/0	0/0	3/97	0/139	0/184
Bulgaria	73/491	6/360	2/569	1/349	0/224
Czech Republic	4/31	7/53	12/68	13/155	17/141
France	0/62	0/50	0/179	0/211	0/220
Lithuania	54/334	3/273	1/173	0/130	0/2
Netherlands	1/640	1/680	2/652	12/646	6/660
Portugal	0/200	0/200	0/0	11/26	7/112
Slovakia	18/203	7/162	1/45	10/68	3/99
Slovenia	1/27	1/30	0/30	0/34	0/52
Spain	0/1376	0/2385	0/5115	4/2529	39/6164
Switzerland	1/1	1/1	1/1	1/1	1/1
United Kingdom	3/83	21/172	0/53	1/38	5/113

E. amylovora and EU legislation

- "Protected Zones"

- Specific additional controls for certain harmful organisms present in the Community or only a risk for restricted areas of the Community.

- All Spain was a protected zone.

❖ **INTENSIVE SURVEYS SINCE 1993:**

- Visual inspections twice a year following an established network.

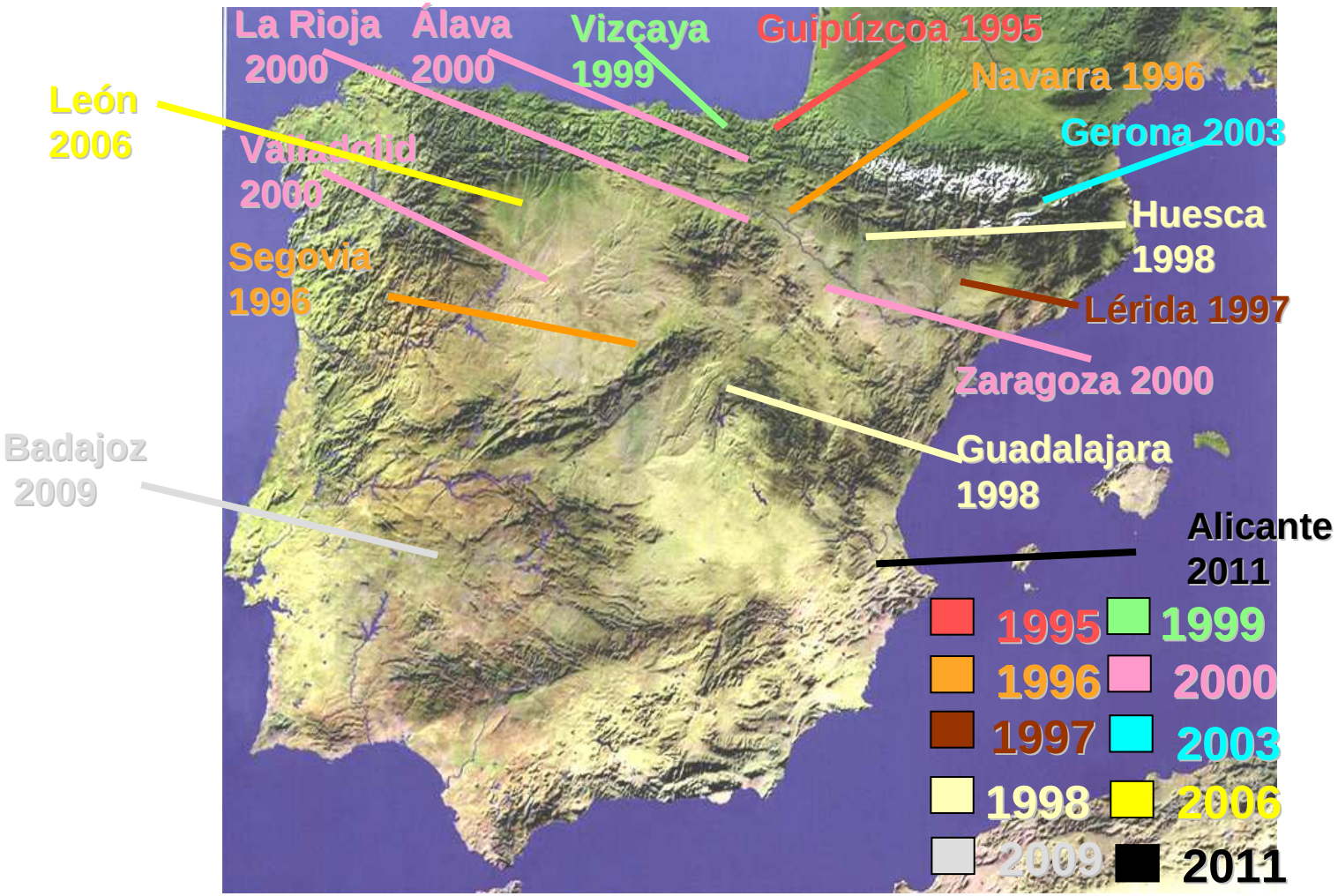
- Laboratory analysis of symptomless plants (from Spanish nurseries and/or imported from EU or other countries).

❖ **SPANISH LEGISLATION SINCE 1999:**

-Compulsory eradication with economic compensation.

-Creation of a buffer zone surrounding outbreaks.

O **Fireblight in Spain: chronicle of a foretold disease that advanced slowly.**



O **The discovered outbreaks were eradicated since 1995 until 2011.**

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LOOKING FOR *E. amylovora* in *SPAIN*

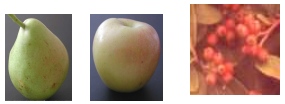
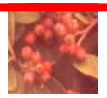
- **Collection of isolates of *E. amylovora* from the different outbreaks.**

- **Diversity of Spanish strains: finding strains with special characteristics.**

- **Tracking of inoculum sources, based on diversity results.**

- **Characterization of a new species of *Erwinia*: *Erwinia pirifloringrans***

HOSTS AND ORIGIN OF *E.amylovora* IN SOME FOCI

YEAR		SPECIES	POSSIBLE SOURCE
GUIPÚZCOA	1995		French foci, plant material
SEGOVIA	1996		Nursery plants
NAVARRA	1996		Guipúzcoa foci, plant material
HUESCA-MADRID	1998		Plant material
GUADALAJARA	1998		Nursery plants
LÉRIDA	1998		Unknown
VIZCAYA	1998		Guipúzcoa foci, plant material
ZARAGOZA-LA RIOJA	1998		Unknown
ÁLAVA-VALLADOLID	2000		Plant material
PALENCIA-BURGOS	2001		Plant material

Polyphasic analysis of Spanish strains of *E. amylovora*

- ✓ **63 Spanish strains from 8 regions and 7 host plants (1995-2001).**
- ✓ **Phenotypic analyses:**

  - API 20E, API 50CH, ATB G-5, API-ZYM and BIOLOG.
- ✓ **Molecular analyses:**

  - MSP-PCR (M13), AFLP (*EcoR*I/*Mse*I, E00/M01-g), RAPD (1281, 1290), PCR-ribotyping (16S-23SrRNA) and PFGE (*Xbal*), plasmid content.

GEOGRAPHIC DISTRIBUTION OF PFGE PATTERNS

PATTERNS	DISTRIBUTION
Pt1	United Kingdom, Central Europe, New Zealand
Pt2	Egypt, Mediterranean countries
Pt3	France, Belgium, Netherlands, Italy
Pt4	United Kingdom, West France

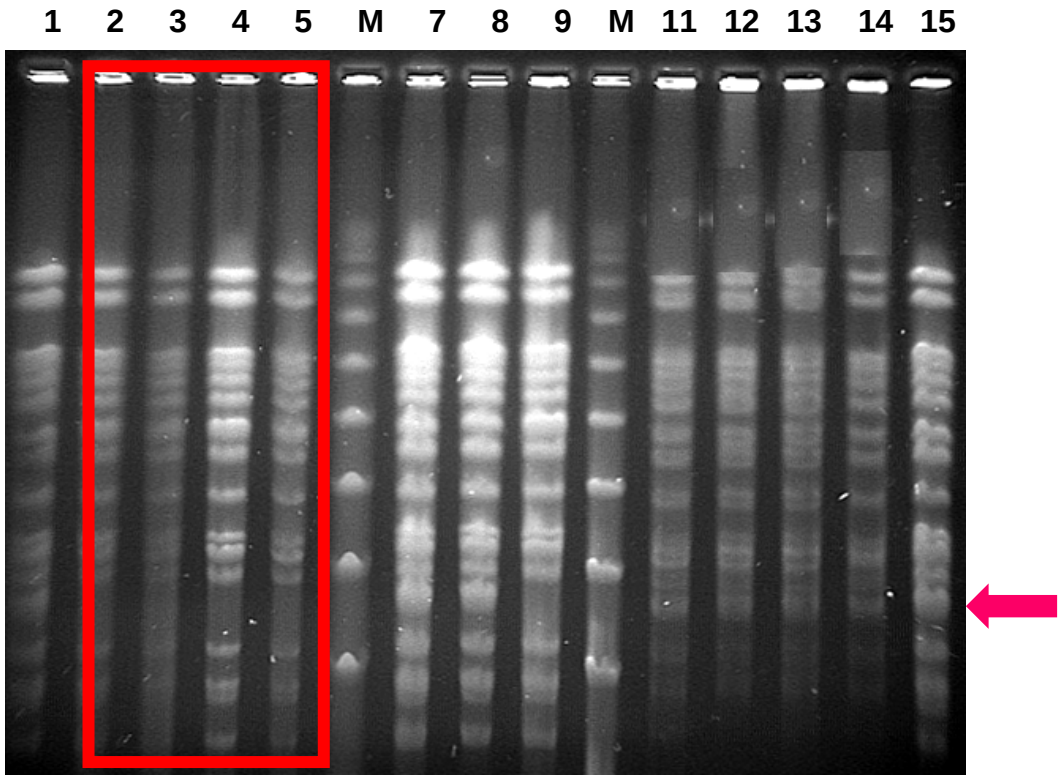
Other Patterns:

Pt5	Bulgary, Israel
Pt6	Italy

According to Jack *et al.*, 2002

Molecular analyses

PFGE



Pt1: 1, Navarra

Pt4: 11-14, Huesca, 15 Lleida

Pt3: 2-5, Guadalajara

Ref.: 7-9

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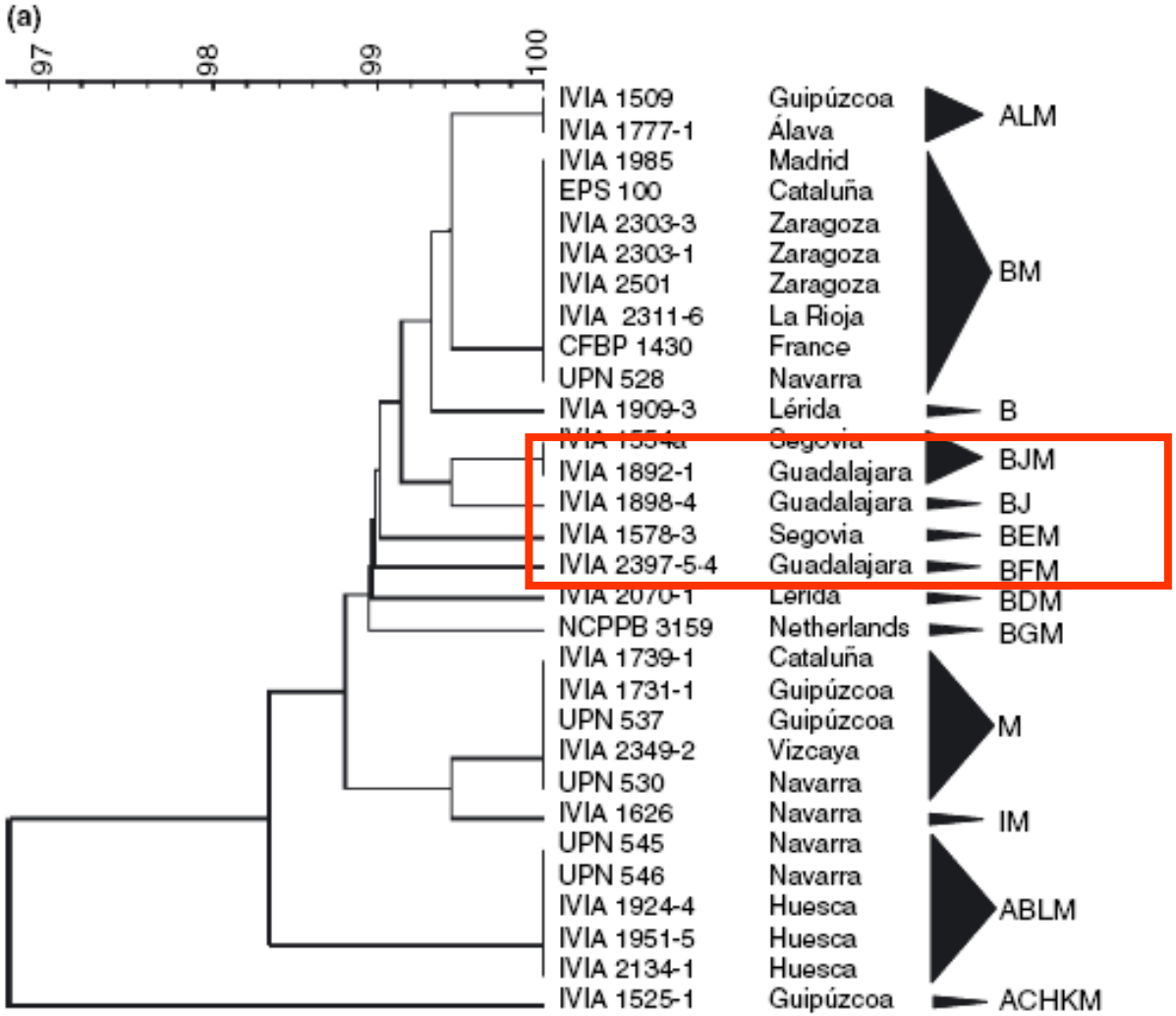
Molecular analyses by AFLP

Typing Spanish *E. amylovora* strains



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ABOUT *E.amylovora* PLASMIDS

- ✓ pEA29 is absent in some Spanish strains but they are fully virulent.
- ✓ A new plasmid (pEI70) has been found in *e. amylovora* strains from different outbreaks in Spain, most of them related to imported plants.
- ✓ This plasmid has also been found in strains from other European countries.
- ✓ The plasmid profile could be employed in epidemiological studies to follow the spread of the disease.

Llop *et al.*, Phytopathol. 96: 900-907, 2007

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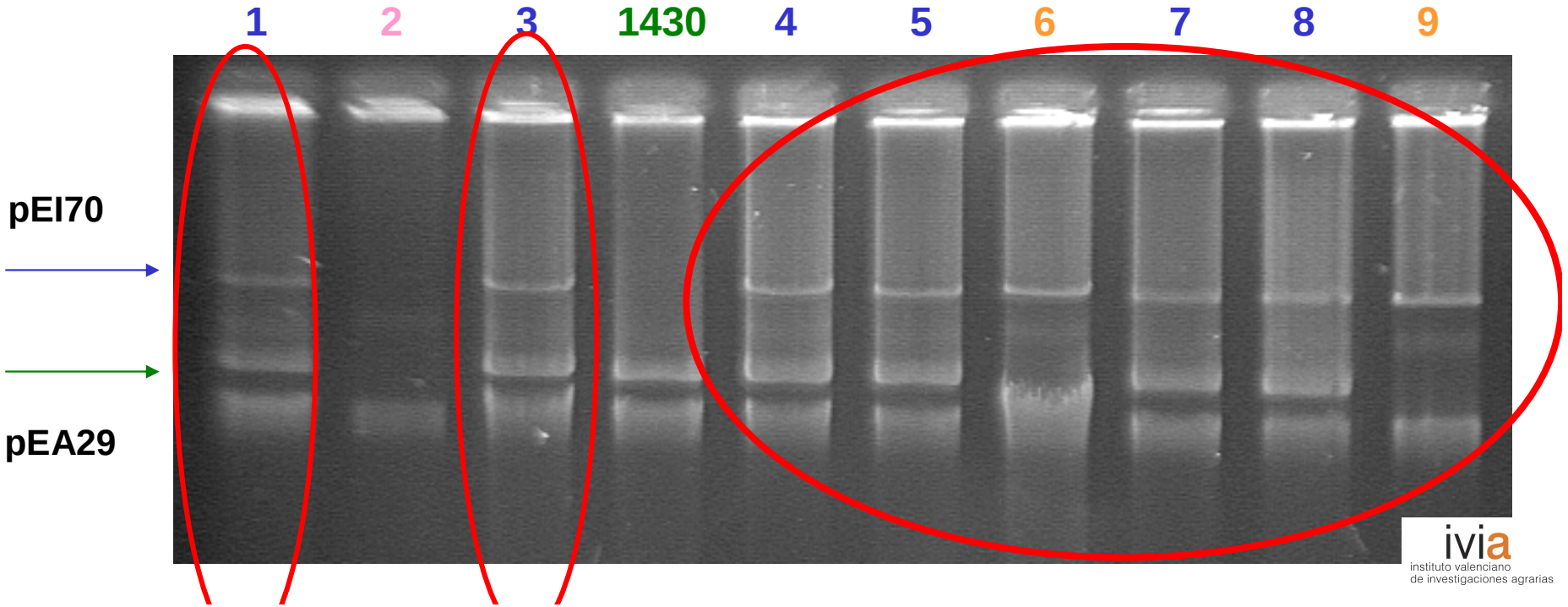
Isolates from outbreaks in Spain without pEA29

- FROM 142 ISOLATES ANALYSED SOME OF THEM CARRY ONE PLASMID OF c. 70KB

- OTHER ISOLATES CARRY BOTH PLASMIDS: pEA29 AND pEI70

- ONE ISOLATE FROM SPAIN HARBOURS NO PLASMIDS



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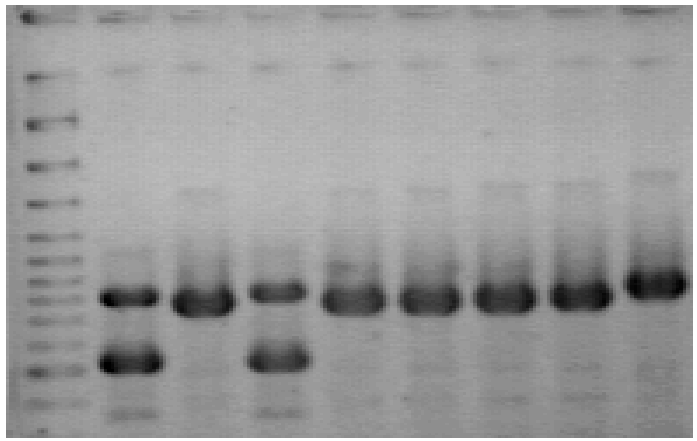
Multiplex analysis of strains from different countries for pEA 29 and pEI 70

PRESENCE OF pEI70 IN EUROPEAN COUNTRIES
ANALYSES OF COLLECTIONS:



	Strains analysed / positive strains
GREECE	15 / 0
AUSTRIA	25 / 0
GERMANY	12 / 0
HUNGARY	10 / 0
BULGARY	4 / 0
USA	7 / 0
CANADA	3 / 0
CHEZK REPUBLIC	2 / 1
FRANCE	41 / 1
NETHERLANDS	4 / 2
ITALY	5 / 3
UK	8 / 2
POLAND	120 / 7 (5.8%)
SPAIN	142 / 19 (13%)
BELGIUM	70 / 65 (92,8%)
IRELAND	14 / 12 (85,7%)
SLOVENIA	(70%)

How to control the Rosaceous plants to prevent introduction, establishment and dissemination of *Erwinia amylovora* in new areas?

- Two Spanish outbreaks were directly related to imported plant material and the EU payed for compensation to the growers.

- Many Spanish outbreaks are indirectly related to introduction of plant material.

- There are many validated methods for routine analyses available in the EU laboratories, that can be used for surveys and analyses of symptomatic and asymptomatic material.

ACKNOWLEDGEMENTS

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T. Temple for LAMP.

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- EPPO Panel of Bacteriology.

- IPPC Panel on Diagnostics.

- Laboratories of Bacteriology and Virology and Immunology (IVIA).

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Many thanks for your attention

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