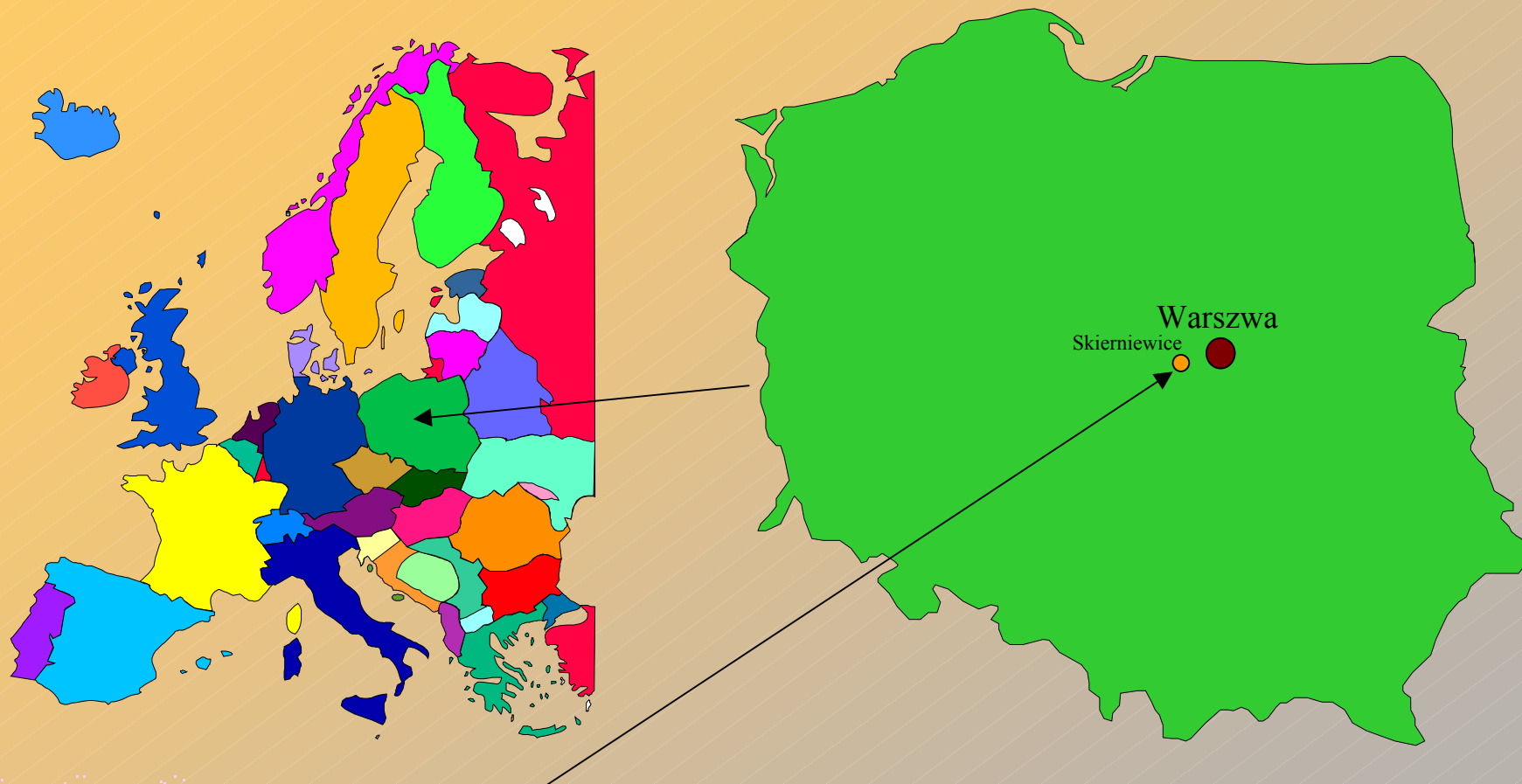


Identification of some economically important pathogens of fruit trees, bushes and ornamental plants

Joanna Puławska, Tadeusz Malinowski, Teresa Orlikowska
Research Institute of Pomology and Floriculture,
Skierniewice, Poland



Research Institute of Pomology and Floriculture
Instytut Sadownictwa i Kwiaciarnstwa
Pomologiczna 18
96-100 Skierniewice
Poland

<http://www.insad.pl/ang/indexang.htm>

Research activities

of Res. Institute of Pomology and Floriculture

- breeding and evaluation of new cultivars
- research on biochemistry and physiology of fruit trees and bushes
- studies on occurrence of plant pests and diseases and development of environment safe methods of their control
- collection and maintenance of genetic resources
- improving fruit quality and extending their postharvest life

- Dept. of Plant Protection
 - Laboratory of Virology (T. Malinowski, M. Kamińska, M. Cieślińska, B. Komorowska, T. Golis, H. Śliwa)
 - Laboratory of Bacteriology (P. Sobiczewski, J. Puławska, K. Kielak, S. Berczyński)

- Dept. of Biotechnology of Ornamental Plants
(T. Orlikowska, K. Wiejacha, A. Marasek, D. Sochacki)

Research activities of Virology Laboratory

many of them in close co-operation with other units

Overview:

- **hosts:** pomology (fruit trees, small fruits) floriculture (ornamentals)
- **viruses:** ACLSV, ASGV, ASPV, ApMV, BRV, PDV, PNRSV, PPV, RBDV, SMoV, TSWV
- **phytoplasmas:** PD-MLO, AP-MLO, AY-MLO,
- **problems:** phytodiagnostics, certification, (thermo, chemo) therapy, characterisation of pathogens, epidemiology, evaluation of resistance (tolerance) to virus diseases, transgenic plants
- **methods:** biological indexing, ELISA, PCR, RT-PCR, IC and SC - RT-PCR, isolation and purification of viruses, preparation of polyclonal and monoclonal antibodies, *in vitro* cultures, cDNA cloning, sequencing ...

List of virus and virus-like pathogens in the collection maintained at Laboratory of Virology

Pathogen or disease name	Akronim	Number of isolates	
		rooted plants	<i>in vitro</i> cultures
<i>Apple chlorotic leaf spot virus</i>	ACLSV	10	6
<i>Apple stem grooving virus</i>	ASGV	10	1
<i>Apple stem pitting virus</i>	ASPV	20	2
<i>Apple mosaic virus</i>	ApMV	4	2
<i>Plum pox virus</i>	PPV	> 6	2
<i>Prune dwarf virus</i>	PDV	9	5
<i>Prunus necrotic ringspot virus</i>	PNRSV	>10	7
<i>Strawberry mottle virus</i>	SMoV	15	1
<i>Raspberry bushy dwarf virus</i>	RBDV	1	1
<i>Raspberry vein chlorosis virus</i>	RVCV	1	1
<i>Raspberry leaf spot virus</i>	RLSV	1	0
<i>Blackcurrant reversion virus</i>	BRV	>30	0
<i>Apple proliferation phytoplasma</i>	AP-MLO	1	0
<i>Pear decline phytoplasma</i>	PD-MLO	1	1
<i>Rubus stunt phytoplasma</i>	RS-MLO	8	2
<i>Aster yellows phytoplasma</i>	AY-MLO	12	0
<i>Apple rubbery wood</i>	ARW	2	0
<i>Pear stony pit</i>	PSP	1	0
<i>Strawberry June yellows</i>	SJY	1	0

Virus and phytoplasma isolates were identified using following methods:

- ELISA with MAbs
- RFLP - analysis
- type specific RT-PCR
- direct sequencing of PCR product
- biological indexing using herbaceous and woody host indicators

Plum pox virus (PPV)

isolate name	original host	type	antiserum	characterised by			GenBank accession number
				RT-PCR-RFLP	serology*	sequencing (CP gene)	
PPV-S	plum	D	+	+	+	+	U27652
PPV-A1N	myrabolan	D		+	+	+	AF33871
PPV-J5a	plum	M	+	+	+	+	-
PPV-L5	plum	D/m		+	+	+	-
PPV-OpL2		M			+		

* ELISA with 6 different MAbs

Library of cDNA fragments representing potential probes for several viruses and virus-like pathogens (phytoplasmas):

Plum pox virus (PPV) – 5 isolates possessing different properties (D, M and intermediate strains), CP gene and fragments of replicase gene

Prunus necrotic ringspot virus (PNRSV) – 8 isolates from 3 phylogenetic groups, CP gene

Apple stem pitting virus (ASPV) – 11 isolates, CP genes with one or two deletions, replicase fragment

Blackcurrant reversion virus (BRV) – cDNA fragments covering > 3000 nt from RNA-2 3'end, including CP gene for at least six isolates from plants showing ETR or RTR symptoms

Apple chlorotic leaf spot virus (ACSLV) – CP gene of SX/2 isolate, smaller fragments of other isolates

Apple stem grooving virus (ASGV), Prune Dwarf virus (PDV) – short fragments of several isolates

Pear decline MLO (PD-MLO) and other phytoplasmas – fragments of 16S rRNA gene

- **Most of potential probes are already available as cloned cDNA.** Some are available as PCR products.
- Based on the collection of plant viruses and virus-like pathogens maintained in laboratory of virology, more probes (PCR products) for other isolates and other plant pathogens could be prepared and offered for common action (preparation of microarrays for detection of plant pathogens).

Research activities of Laboratory of Bacteriology

Overview

- **hosts:** fruit trees, ornamental plants
- **organisms:** tumorigenic *Agrobacterium*, rhizobia, *Erwinia amylovora*, *Pseudomonas syringae*
- **problems:** phytodiagnostics, detection and characterization of pathogens, phylogeny, biodiversity, ecology of pathogens, epidemiology, control,
- **methods:** pathogenicity tests, PCR, RAPD - PCR, PCR - RFLP, sequencing.

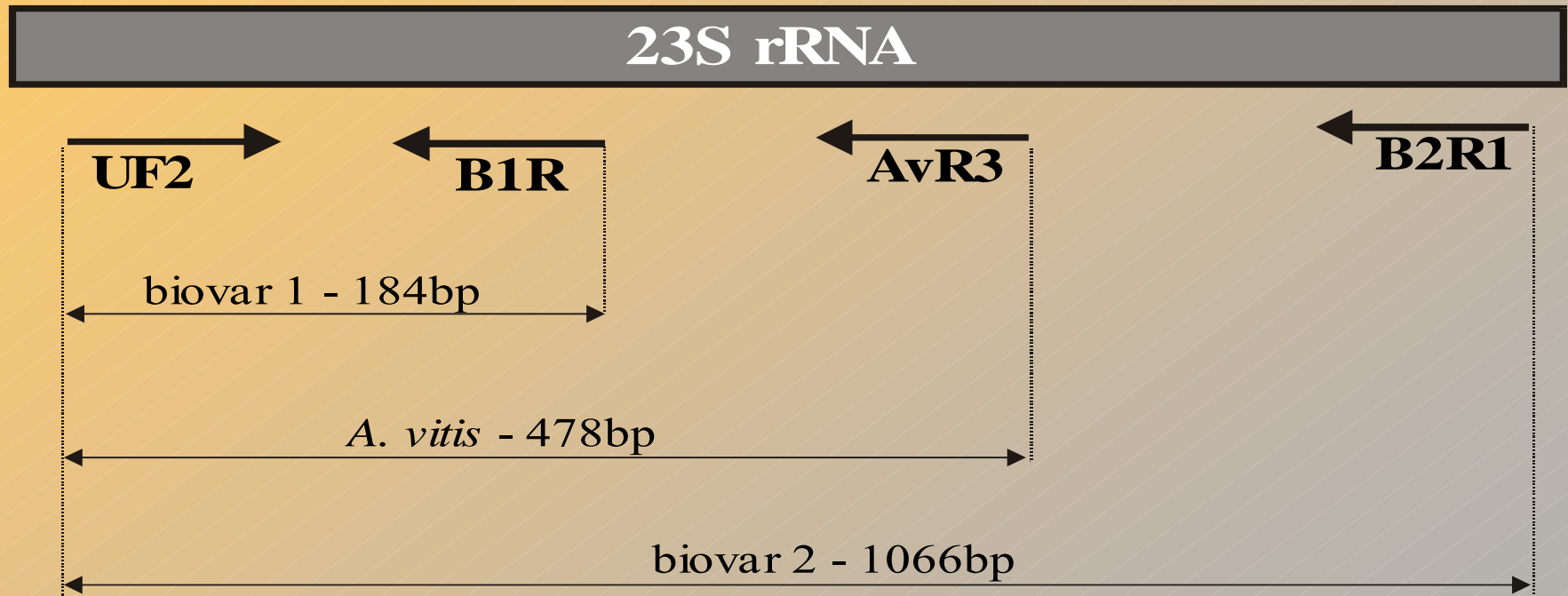
Collection of bacterial strains:

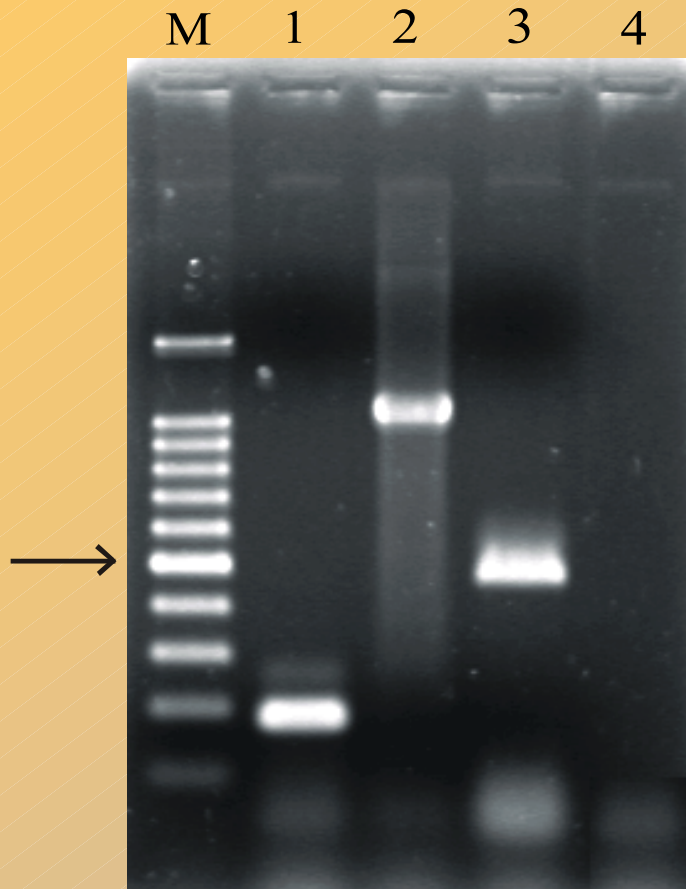
- 102 strains of *Agrobacterium*
 - isolated from various hosts (fruit trees, fruit shrubs, ornamental plants, crops...)
 - in different geographical origins (Poland, France, Greece, Israel, Hungary, Australia, USA, Japan...)
- rhizobia
 - 20 strains of diverse genera (*Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*)

Collection of bacterial strains:

- 93 *Erwinia amylovora*
 - isolated from various hosts (apple, pear, quince, hawthorn, mountain ash)
 - in different geographical origins
- Other plant pathogenic bacteria
(*Pectobacterium chrysanthemi*, *Pseudomonas*, *Xanthomonas*)

Identification of biovar 1, 2 *Agrobacterium* and *A. vitis* with multiplex PCR





Multiplex PCR with primers UF2 + B1R + B2R1 + AvR3 and DNA of:

1. *A. tumefaciens* B6 (biovar 1)

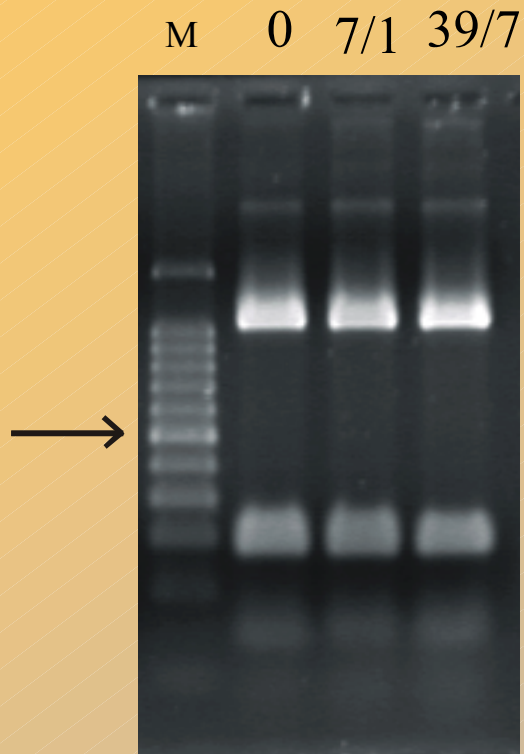
2. *A. radiobacter* LMG 150 (bv 2)

3. *A. vitis* LMG 8750

4. *A. rubi* LMG 156

M – molecular weight marker –
100 bp ladder

Primers for *A. rubi* identification are designed and at present tested for their specificity

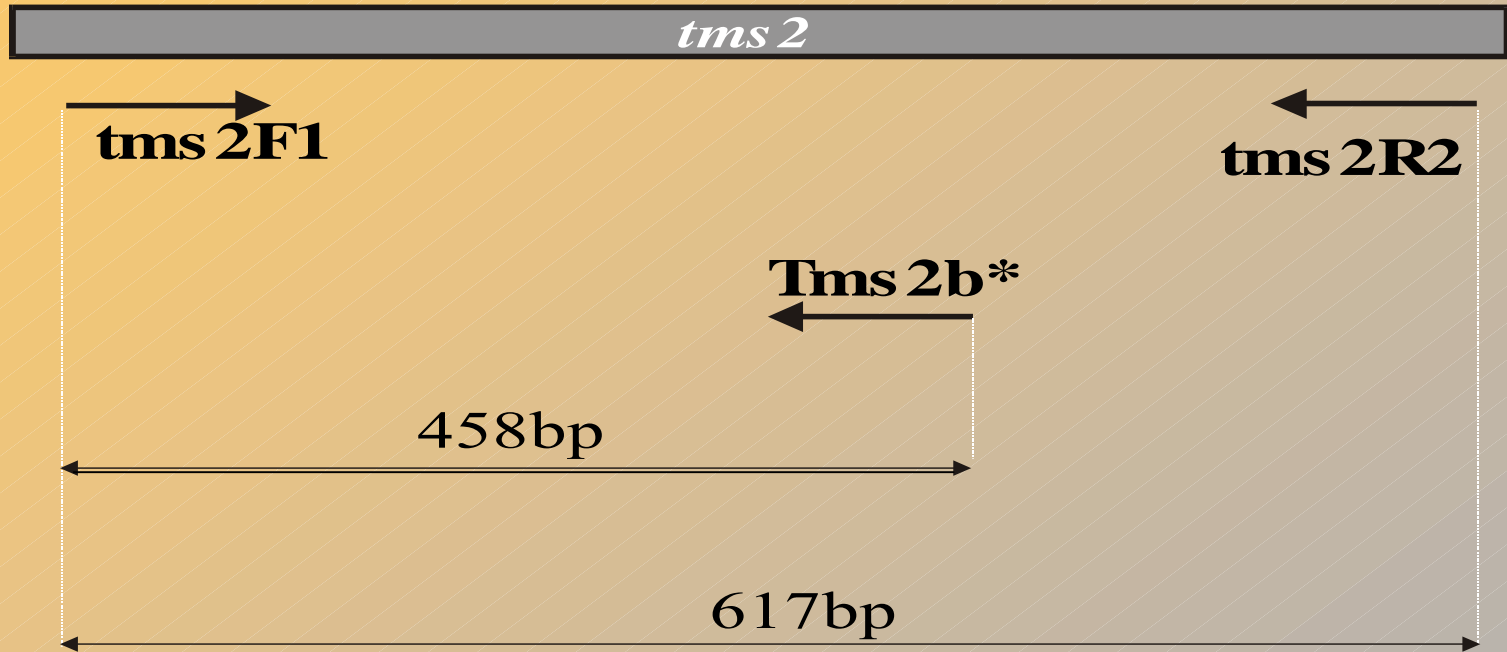


Atypical strains:

- 0, 7/1, 39/7

- Ch11, Ch12 (???)

Identification of tumorigenic *Agrobacterium* strains



* Sachadyn & Kur (1997) Acta Microbiol. Polonica

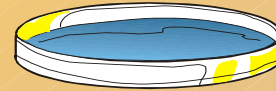
Detection of tumor-inducing *Agrobacterium* in soil



Soil sample



Incubation of soil sample water suspension on selective medium (1A+2E)* for 3 days



Bacterial cell lysis in four cycles of freezing and boiling followed by Proteinase K treatment

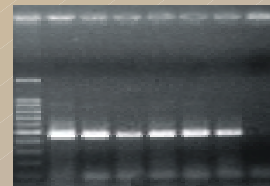


DNA purification on Genomic DNA Prep Plus columns

Semi-nested PCR with primers complementary to T-DNA

It is possible to detect as few as 1-2 cells of tumorigenic *Agrobacterium* in gram of artificially inoculated soil sample.

PCR - product 458bp



* medium of Brisbane&Kerr (1983) J.Appl. Bacteriol. with own modification

Detection of *Erwinia amylovora*



- **Specificity of primers***
- **Influence of pesticide residue on detection by PCR****

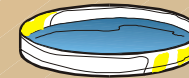
Bacteria on/in plant



Washing of plant sample in buffer



Incubation of washings on SNA medium



DNA isolation (100°)



DNA purification on Genomic DNA Prep Plus columns

PCR with primers complementary to 23S rDNA*



565bp



* Maes et al. (1996) Plant Pathology

** Puławska et al. (1997) Med. Fac. Landbouw. Univ.

** Puławska & Sobiczewski Acta. Hort (in press)

Research activities of Dept. of Biotechnology of Ornamental Plants Overview

- **hosts:** ornamental trees, shrubs and flowers
- **organisms:** *Phytophthora citricola*, *P. cryptogea*, *P. cinnamomi*,
- **problems:** detection and identification, genetic diversity
- **methods:** RAPD, ISSR - PCR, ITS - PCR, RFLP, sequencing

Collection of isolates:

- 46 isolates of fungi belonging to genus *Phytophthora*:
 - *P. ramorum*, *P. citricola*, *P. cryptogea*, *P. cinnamomi*
isolated from various hosts in Poland

- building of collection of isolates
- phenotypic characterization
- testing of primers existing in literature
- development of new DNA - based markers for pathogens identification, detection and differentiation