

# Diversity in *Erwinia amylovora* virulence and development of detection method of pathogen in plant material

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*Erwinia amylovora*, the causal agent of fire blight, is considered to be a homogeneous species based on physiological, biochemical, phylogenetic and genetic analysis. However, *E. amylovora* strains differ in virulence.

The aim of our study was to compare the virulence of 6 *E. amylovora* strains isolated in Poland from various hosts and regions with 2 strains isolated in USA as well as analysis of its genetic diversity.

## Analysis of virulence of *E. amylovora* strains

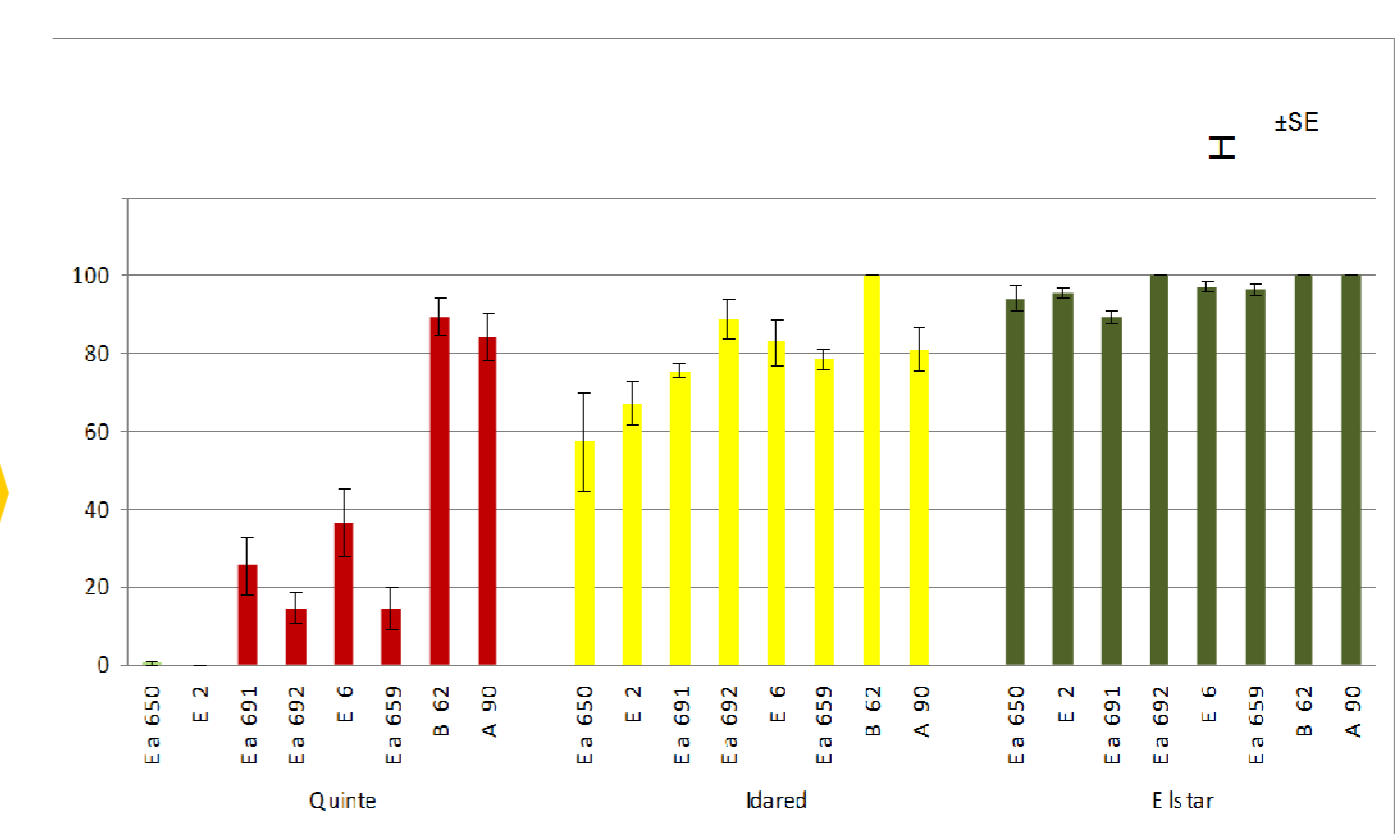
Strains:

659 – *Malus* (1986), Poland  
 691 – *Malus* (1998), Poland  
 E6 – *Pyrus* (2000), Poland  
 692 – *Sorbus* (1998), Poland  
 650 – *Crataegus* (1983), Poland  
 E2 – *Crataegus* (2000), Poland  
 A90 – *Malus*, USA  
 B62 – *Malus*, USA

Strains were used for inoculation of apple trees cvs. **Idared** (susceptible), **Elstar** (middle susceptible) and **Quinte** (resistant)



The virulence was measured 2, 4 and 6 weeks after inoculation.

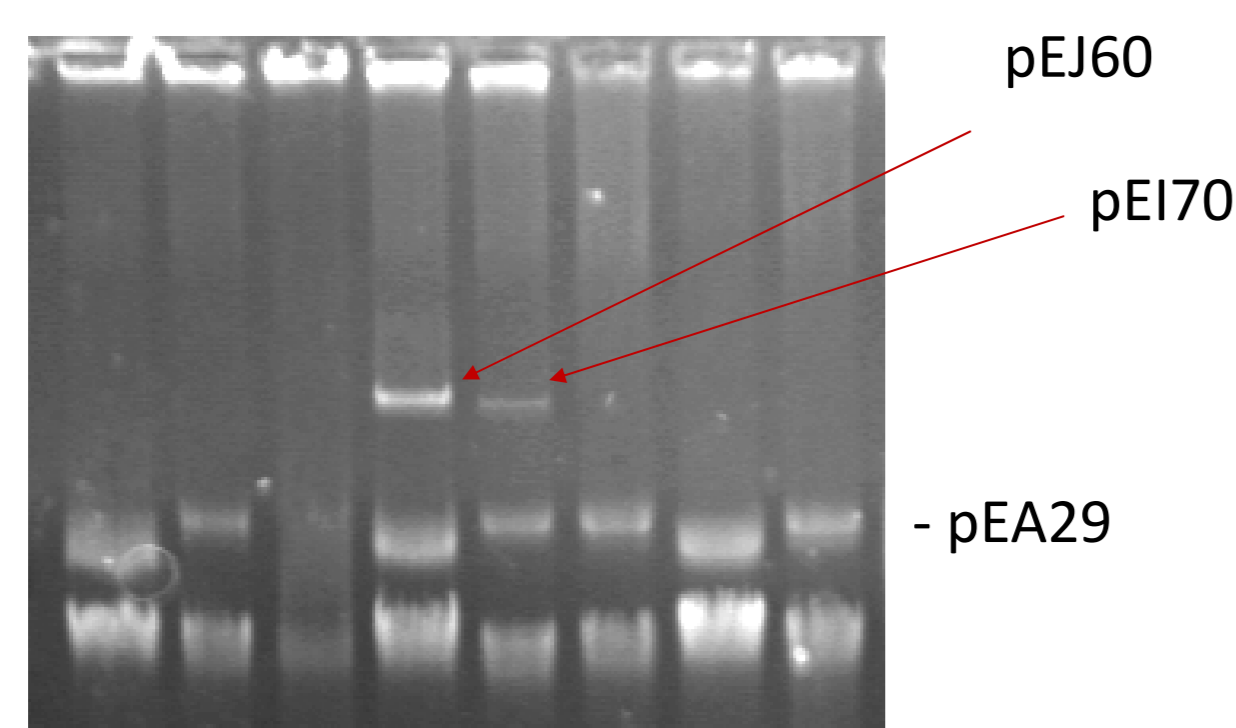


The virulence of tested *E. amylovora* strains— expressed as a percentage of shoot necrosis in relation to entire length of shoot measured six weeks after inoculation.

## Analysis of genetic diversity of *E. amylovora* strains

### Plasmid profiles

650 659 691 692 E2 E6 B62 A90



All strains possessed plasmid pEA29. Plasmid pEI70 (Llop et al. 2008) was found in strain E2 while new plasmid pEJ60 (Puławska, unpublished) in strain 692.

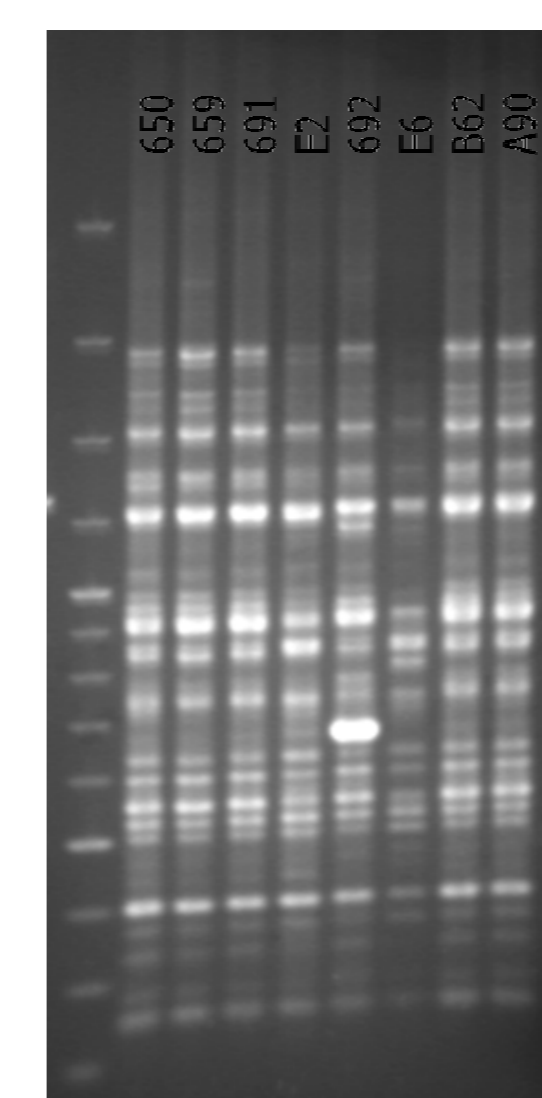
### Analysis of sequences of genes related to pathogenicity

The sequences of genes:

- = *hrpN* (Jock and Geider, 2004)
- = *dspA/E* (Giorgi and Scortichini, 2005)
- = *amsB* (Bereswill et al., 1995)

were identical

### RAPD



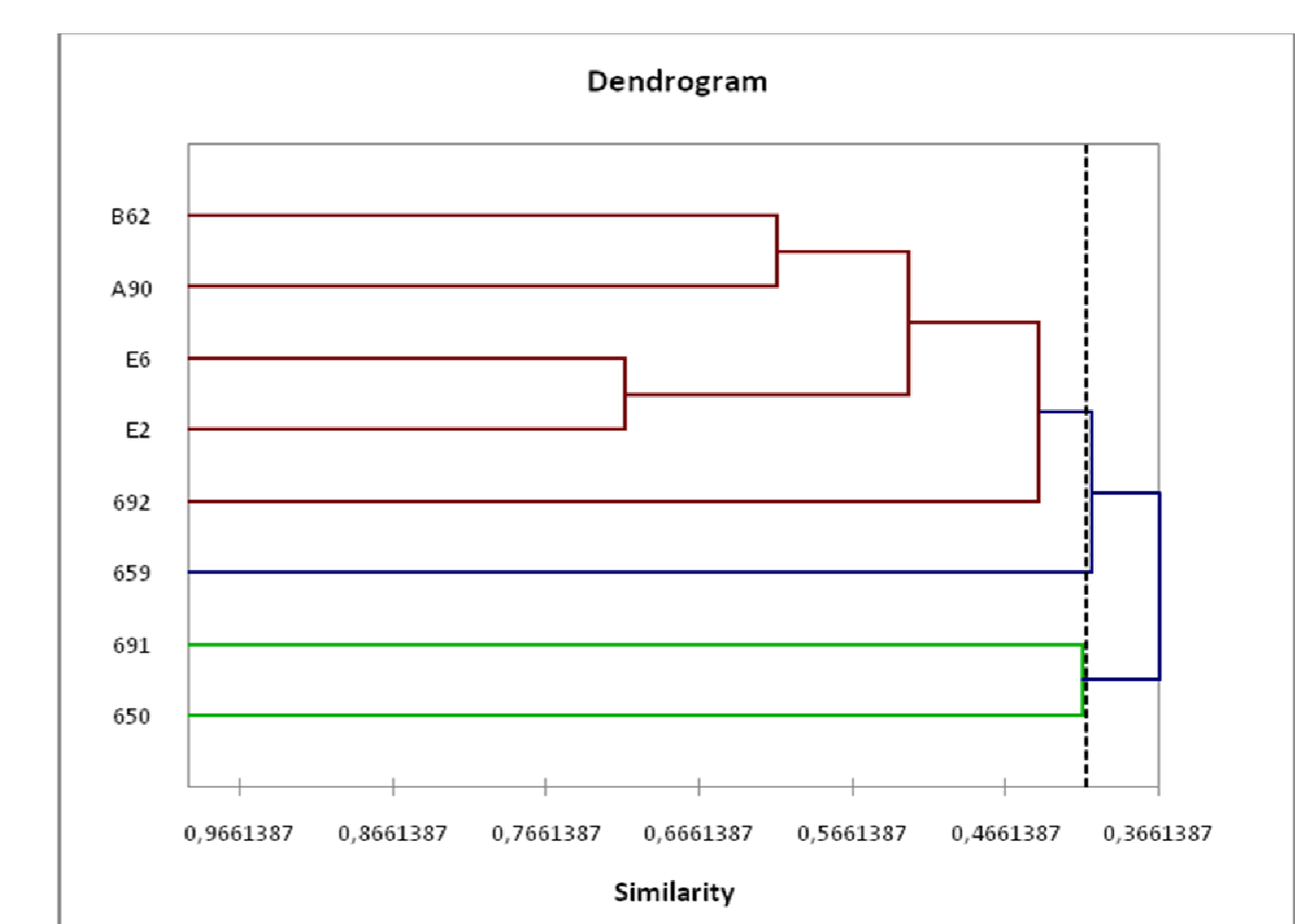
OPAR03

RAPD analysis with 19 different random primers showed almost no differences between strains.

### AFLP

EcoRI + PstI  
 EcoRI + MseI

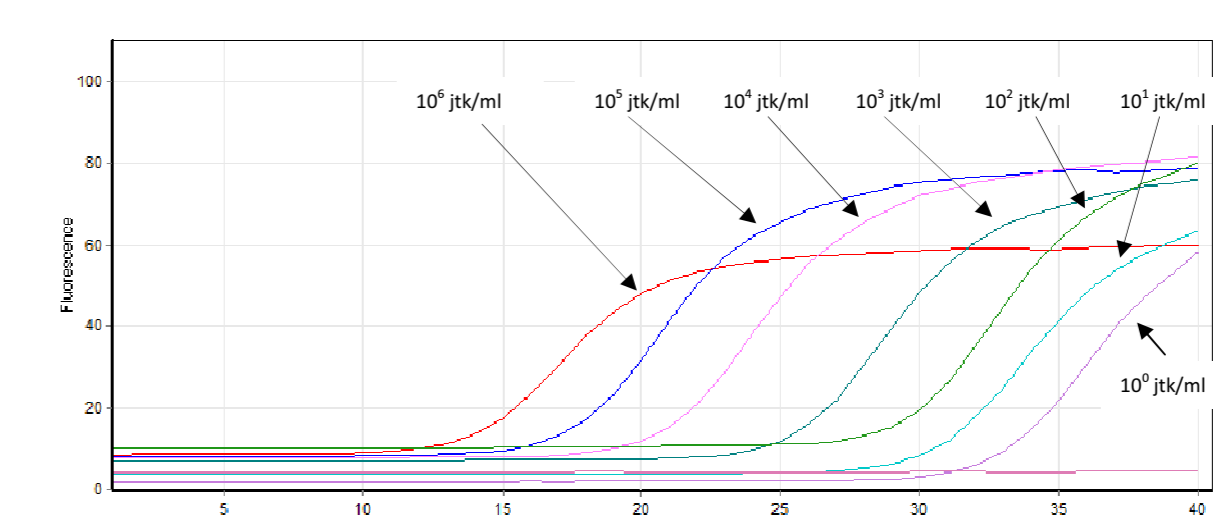
6 out of 11 selective primers allowed for strain differentiation



There was no correlation observed between AFLP patterns and virulence was found.

## Detection of *E. amylovora* in plant material

Out of three *E. amylovora* detection methods tested: i/ classical microbiological method, ii/ standard PCR and iii/ real-time PCR with primers complementary to ubiquitous plasmid pEA29, the real-time PCR technique was found as the most specific and sensitive one. Real-time PCR technique allowed for detection less than 10 cfu/ml of *E. amylovora* cells.



### Summary and conclusion:

- All strains tested differed in virulence.
- The highest diversity between virulence was observed on cv. Quinte. These results indicate both the usefulness of cv Quinte for the selection of strains of *E. amylovora* for screening tests intended to evaluate breeding material, in terms of resistance/susceptibility to fire blight.
- Strains B62 and A90 were the most virulent.
- All strains tested were very similar genetically.
- AFLP – the only technique which allowed for differentiation of Ea strains no correlation between AFLP patterns, geographical origin, host plant or virulence was found.
- Selection of strain(s) with very high virulence (amount of disease and host range) is very important in breeding for resistance to fire blight.
- Real-time PCR technique was found as the most specific and sensitive for detection of *E. amylovora*.