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Characterization of Yugoslavian *Erwinia amylovora* Isolates Originating from Apple Trees Comparing with Other Strains of the Pathogen

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Pathogenicity, cultural and biochemical characteristics of the *Erwinia amylovora* strins of various origin were ivestigated. Six of them originated from apple (Yugoslavia and Canada), and four from pear and quince trees (England and Yugoslavia). No differences were observed in any property among the strains investigated.

Keywords: *E. amylovora,* apple strains, cultural characteristics, pathogenicity.

From 1989 when the *E. amylovora* was detected for the first time in Yugoslavia up to 1995 the pathogen prevailed on pear and quince as well as on mespil trees (Arsenijević et al., 1991; Panić and Arsenijević, 1993). Its occurrence in apple orchards was rear of the weak intensity (Panić and Arsenijević, 1993, 1996).

However, during 1995 and 1996 the first fire blight symptoms suddenly outbreaked and spread in numerous district and orchards where apple cultivars were grown (Arsenijević et al., 1996). So behaviour of the pathogen and the strong infection of the apple trees six years after the first outbreak and spread of the fire blight disease in pear and quince orchards, we thought, may be explained by the heterogeneous population of the *E. amylovora* bacterium which probably exists in Yugoslavia.

Therefore, it was a main reason to investigate pathogenicity and bacteriological characteristics of the *E. amylovora* pome strains isolated in Yugoslavia and abroad (England and Canada) from apple, pear and quince trees.

Materials and Methods

The strains used for investigation and their preliminary identification

From the diseased apple trees many strains of *E. amylovora* were isolated in Yugoslavia by usual procedure (Lelliott and Stead, 1987; Klement et al., 1990). Five of them (Ea-4, Ea-72, Ea-75, Ea-83, Ea-95) originated from different localities and apple cultivars were chosen for further study. As the check strains five *E. amylovora* isolates were used: two from England (NCPPB 595 isolated from pear by Dr. J. E. Crosse in 1958 and 5378 from quince isolated in 1983; both strains were received from Dr. Steve Roberts, HRI, Wellesbourne), one from Canada (E81004A received from Dr. W. G. Bonn,

Harrow, Ontario isolated in 1981 from apple) and two from Yugoslavia (Ea-205 and Ea-311 isolated in 1996 from pear and quince, respectively). All the strains used were stored on NAG (Nutrient agar + 2% glycerol) slopes at 4 °C.

Preliminary identification of the strains studied was carried out using the following tests: Gram differentiation, tobacco hypersensitivity, fluorescence on King's medium B, slide agglutination test and artificial inoculation of immature pear fruits. The immature pear fruits (cv. Williams) were inoculated by pricking using bacterial suspension 10^7 cfu/ml. Slide agglutination test was also performed using conventional procedure and antisera prepared by authentic *E. amylovora* apple strain (1430), obtained from Dr. J. P. Paulin (Station de Pathologie Végétale, Angers, Beaucouzé, France). All tests were carried out according to Klement et al. (1990).

Plant inoculation and cultural and biochemical properties

In order to check the pathogenicity of the strains investigated herbaceous and woody plants were used. The leaves of black night-shade (*Solanum nigrum* L. Mill.), Pelargonium, simpson weed (*Datura stramonium* L.) and tomato were inoculated according to standard procedure (Klement, 1963).

In pathogenicity tests immature apple fruits, quince, plum, sweet cherry, *Sorbus,* Japanese apple fruits were used. Apple, pear, quince, firethorn raspberry and blackberry shoots were also studied. For both, shoots and immature fruits inoculation the bacterial suspension (10^7 cfu/ml) were introduced by pricking into the plant tissues (Klement et al., 1990). The inoculation fruits and shoots were kept in a moisture chambers for three days, and then kept at laboratory conditions.

Growth characteristics of the *E. amylovora* strains were examined on sucrose nutrient agar (SNA) and on Crosse-Goodman (CG) selective medium (Lelliott and Stead, 1987). Tolerances to 5% and 7% NaCl were investigated according to Fahy and Persley (1983; loc cit. Arsenijević, 1997).

Glucose metabolism, oxidase and catalase activity, starch and Tween 80 hydrolysis, indole, H_2S and NH_3 production, methyl red and VP tests were also studied (Lelliott and Stead, 1987; Klement et al., 1990).

Carbohydrates utilization and alkali production from organic acid were analysed according to Klement et al. (1990) and Kim et al. (1996) *(Table 1).* The solid media were streaked using 24-hr-old bacteria cultured on nutrient agar. All tests were carried out at 26 °C.

Results

Preliminary identification characteristics and pathogenicity

No differences among the investigated strains were noticed. All *E. amylovora* strains were asporogenous, gram-negative and rod-shaped. On King's medium B no green fluorescent pigment appeared. Tobacco hypersensitivity and immature pear fruits tests were positive as well as the slide agglutination test.

Table 1

Biochemical and physiological properties of the *E. amylovora* strains investigated

Test	Apple strains (Ea-4, Ea-72, Ea-75, Ea-83, Ea-95, E 81004A)	Pear strains (NCPPB 595, Ea-205)	Quince strains (5378, Ea-311)
O/F test on glucose			
aerobic	+	+	+
anaerobic	+	+	+
Hydrolysis of:			
starch	–	–	–
tween 80	–	–	–
Production of:			
indole	–	–	–
NH_3	–	–	–
H_2S	–	–	–
VP test	+	+	+
Methyl red	–	–	–
Activity of:			
oxidase	–	–	–
catalase	+	+	+
Utilization of:			
glucose	+	+	+
fructose	+	+	+
sucrose	+	+	+
xylose	+	+	+
lactose	–	–	–
mannitol	+	+	+
sorbitol	+	+	+
inositol	+	+	+
glycerol	+	+	+
acetate	+	+	+
citrate	+	+	+
D (–) tartrate	+	+	+
cis – aconitic acid	+	+	+

+ positive; – negative result

All investigated *E. amylovora* strains incited HR on Pelargonium, black nightshade, simson weed and tomato leaves. On pome and stone immature fruits artificially inoculated, they caused tissue necrosis and bacterial oozing formed on pear (cv. William's), apple (cv. Gloster), quince (cv. Leskovacka), plum (cv. Stanley), sweet cherry (cv. Sue) and *Sorbus* (domestic spontaneous population), but not on Japanese apple fruits.

Strong tissue necrosis followed by fire blight symptoms appeared on inoculated shoots of pear (cv. William's), apple (cv. Jonathan), quince (cv. Leskovacka) and firethorn (*Pyracantha coccinea* L.). No symptom was noticed on raspberry (cv. Willamette) and blackberry (cv. Thornfree) shoots artificially inoculated.

The reisolation of the pathogen using necrotic tissues taken from the shoots infected were successful. Slide agglutination test carried out with reisolates obtained was positive.

Bacteriological characteristics of the strains investigated

On sucrose nutrient agar (SNA) the colonies were round, cream, large, convex and mucoid indicated levan formation. After 2–3 days of the development on Crosse-Goodman (CG) selective medium the colonies were pearly-white, high-domed, crateorous and mucoid. All strains investigated developed in a liquid medium with 5% NaCl, but not in a medium with 7% NaCl *(Table 1).*

The strains were oxidase negative and catalase positive; starch and tween 80 hydrolysis as well as the methyl-red test and hydrogen sulphide production from cysteine were negative; VP test (acetoin production) was positive *(Table 1).*

Lactose metabolism was negative and glucose, fructose, sucrose, xylose, mannitol, sorbitol, inositol and glycerol were positive *(Table 1).* Alkali production from acetate, citrate, D (–) tartrate and cis-aconitic acid was positive *(Table 1).*

Discussion

First appearence of the *E. amylovora* pathogen in Yugoslavia was observed in the middle of 1989 (Arsenijević et al., 1991; Panić and Arsenijević, 1993, 1996). Many diseased pear and quince trees were eradicated because fire blight spread quickly and appeared in numerous orchards and localities, with the tendency of further expansion. In 1993 the high degree of infection was also noticed on mespil in many districts. Up to 1995 the disease on apple trees was sporadic without strong infection. However, during 1995, 1996 and 1997 the fire blight symptoms outbraked suddenly and spread in numerous orchards and localities where various apple cultivars were growing (Arsenijević et al., 1996).

Too late and strong infection on many apple cultivars six years after the first disease appearance (1989) on pear and quince susceptible cultivars, observed in Yugoslavia was difficult to explain. Therefore, it was the main reason for parallel investigation of the *E. amylovora* strains originating from apple, pear and quince trees isolated in Yugoslavia, England and Canada *(Table 1).* As it was shown neither cultural nor biochemical differences were noticed among the strains investigated *(Table 1).* With regards to the pathogenicity all isolates induced HR on herbaceous plants. In immature fruits tissue test all pome isolates caused necrotic lesions with exudate oozing. No differences were also noticed in degree of the pathogenicity to apple, pear, quince and firethorn in shoot inoculation test. On raspberry and blackberry shoots artificially inoculated, no fire blight symptoms caused by any isolate studied was observed.

In the USA a group of specific *E. amylovoro* strains were isolated from raspberry and blackberry tissues and they were classified as different *Rubus* strains (Starr et al., 1951; Ries and Otterbacher, 1977). Norelli et al. (1984) reported that the *E. amylovora* strains from the *Rubus* species are host specific parasiting only *Rubus* plants but not

Malaceae. Recent investigations carried out in Belgium (Garbeva et al., 1996) pointed out also that *E. amylovora* strains originated from the *Rubus* plants were unable to cause fire blight symptoms on malaceous seedlings and shoots.

Judging on the results obtained it can be concluded that all the investigated strains isolated in Yugoslavia, Canada and England from pome fruits (apple, pear and quince) showed homogenous characteristics in all test performed *(Table 1).* The homogeneity among *Erwinia amylovora* strains originating from pomaceous and ornamental plants was registered by Vantomme et al. (1982), Shoeib et al. (1987), Brown et al. (1996), Kim et al. (1996).

Therefore, the *E. amylovora* isolates responsible for the strong infection of the apple trees in Yugoslavia does not consist a special strain adapted to Yugoslavian conditions and the apple cultivars growing in this area.

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