Phenotypic Diversity of *Erwinia amylovora* in Bulgaria

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Fifty-one strains of *Erwinia amylovora* isolated from nine host plants in Bulgaria were characterized phenotypically and identified by the API 20E and BIOLOG system. The identification was confirmed by PCR amplification of a specific region of the plasmid pEA29 and the genome *ams* region. The phenotypic diversity of the strains was studied on the basis of their API 20E and BIOLOG metabolic profiles, as well as of their SDS-PAGE protein profile. Metabolic diversity among the strains was established, but no connection with the origin of the strains was revealed. The Bulgarian strains showed API 20E metabolic profiles not found in previous studies of *E. amylovora*. The strains formed a homogenous group on the basis of their protein profiles. All the strains were sensitive to the antibiotics streptomycin, tetracycline and oxytetracycline. This study was an initial step towards an investigation of the diversity and evolution in the Bulgarian population of *E. amylovora,* and it was the first characterization of *E. amylovora* strains isolated from different host plants in the period 1995–2005 in Bulgaria.

Key words: Erwinia amylovora, Phenotypic Diversity, PCR

Introduction

The characterization of host range and strain diversity of *Erwinia amylovora* – the causal agent of the fire blight, is important in plant quarantine, selection for disease resistance, identifying possible sources of infection, distinguishing strain groups and revealing the relations among strains (Momol and Aldwinckle, 2000). Several studies have indicated that strains of *E. amylovora* form a homogenous group. In the past, no characteristics have been found that could distinguish strains of different geographical origins, or strains that have been isolated either from different host plants or at different times (Vanneste, 1995; Momol and Aldwinckle, 2000). The biochemical characterization of *E. amylovora* strains by the API 20E system revealed a great homogeneity in their API 20E profiles (Mergaert *et al*., 1984; Vantomme *et al*., 1986; Donat *et al*., 2005). A few data concerning the biochemical diversity of *E. amylovora* determined by the BIOLOG system are available (Olamendi, 2005). Zarnowski *et al*. (2002) have studied the natural diversity among *E. amylovora* isolates on the basis of total cellular protein and fatty acid patterns. The fatty acid profiles demonstrated more clearly the variation between the isolates.

Several articles characterized *E. amylovora* strains from some European countries either phenotypically or genotypically (Momol *et al*., 1997; Zhang and Geider, 1997; Jock *et al*., 2002; Keck *et al*., 2002). Only a few papers have treated the distribution and the characterization of the pathogen in Bulgaria (Bobev, 1990; Bogatzevska and Kondakova, 1994; Garbeva *et al*., 1996; Bobev *et al*., 1999). The diversity at different levels among the Bulgarian strains of *E. amylovora* has not been studied.

This study represents the initial step in the investigation of the diversity and the evolution of the Bulgarian population of *E. amylovora*. This paper reports on the first characterization of *E. amylovora* strains isolated from different host plants in Bulgaria. It aims to characterize and analyze the occurrence and phenotypic diversity of the *E. amylovora* population in Bulgaria.

Material and Methods

Bacterial strains

Fifty-three presumptive *E. amylovora* strains isolated by a standard procedure (Jones and Geider, 2001) from different parts of the nine host plants with typical symptoms of fire blight were used in this study (Table I). Two of them (marked as 1C and 2C) were isolates from Serbia and were kindly provided by Assoc. Prof. I. Kiryakov (Dobroudja Agricultural Institute in General Toshevo).

Media and culture conditions

King's medium B (King *et al*., 1954) was used for the isolation of the strains. The morphology of colonies was examined on sucrose nutrient agar (Billing *et al*., 1961) and MM2Cu medium (Bereswill *et al*., 1998). The mucoid growth and pigmentation was examined on YDC medium (Schaad, 2001). LB (Merck, Darmstadt, Germany) broth was used for cultivation of the strains for SDS-PAGE and DNA isolation (Sambrook *et al*., 1989). The strains were maintained on potato dextrose agar (Oxoid Ltd, London, UK). The cultivation was at 26 °C for 16 to 48 h depending on the analysis performed.

Characterization and identification procedures

The strains were tested for pathogenicity, and were characterized morphologically and physiologically by the procedures described in our previous work (Atanasova *et al*., 2005). The biochemical characterization and identification of the strains was carried out by two miniaturized systems – API 20E (BioMerieux, Marcy-l'Etoile, France) and BIOLOG (BIOLOG Inc., Hayward, CA, USA), according to the instructions of the manufacturers. The BIOLOG metabolic fingerprint patterns of the strains analyzed were compared using the MicroLog™ version 4.01B database software supplied. The type strain of *E. amylovora* (ATCC 15580) was used as a control.

Isolation of DNA

Chromosome and plasmid DNA were isolated and purified by a DNA isolation kit [Scientific Technological Service (STS) Ltd, Sofia, Bulgaria] according to the instruction of the manufacturer.

Table I. Source and year of isolation of *E. amylovora* strains.

Strain designation	Host plant	Year of isolation
1	*Pyrus* sp.	1995
2	*Pyrus* sp.	1995
3	*Pyrus* sp.	1995
4	*Pyrus* sp.	1995
5	*Pyrus* sp.	1995
6	*Pyrus* sp.	1995
7	*Pyrus* sp.	1995
8	*Pyrus* sp.	1995
9	*Pyrus* sp.	1995
10	*Pyrus* sp.	1995
11	*Malus* sp.	1995
12	*Malus* sp	1996
13	*Pyrus* sp.	1990
14	*Aronia melanocarpa*	2004
15	*Aronia melanocarpa*	2004
16	*Pyrus* sp.	1997
17	*Pyrus* sp.	1997
18	*Pyrus* sp.	1997
19	*Pyrus* sp.	1997
20	*Pyrus* sp.	1997
21	*Pyrus* sp.	1997
22	*Cydonia* sp.	2002
23	*Cydonia* sp.	2003
24	*Cydonia* sp.	2004
25	*Pyracantha coccinea*	2003
26	*Pyracantha coccinea*	2004
27	*Pyracantha coccinea*	2005
28	*Cotoneaster integerrimus*	2005
29	*Cydonia* sp.	2004
30	*Pyrus* sp.	1999
31	*Cydonia* sp.	2004
32	*Pyrus* sp.	1999
33	*Pyrus* sp.	1999
34	*Pyrus* sp.	2002
36	*Crataegus* sp.	1999
39	*Malus* sp.	2000
40	*Malus* sp.	2001
42	*Malus* sp.	2002
44	*Malus* sp.	2003
49	*Malus* sp.	2000
51	*Malus* sp.	2000
52	*Malus* sp.	2002
54	*Malus* sp.	2003
55	*Malus* sp.	2004
236	*Malus* sp.	2001
237	*Fragaria moshata*	1999
238	*Fragaria moshata*	1999
244	*Fragaria ananassa*	2002
245	*Fragaria ananassa*	2003
246	*Pyrus* sp.	2002
247	*Fragaria moshata*	1999
1C	*Pyrus* sp.	–
2C	*Pyrus* sp.	–

PCR

PCR was performed in a thermal cycler (Mastercycler Eppendorf) using two pairs of primers (Invitrogen Life Technologies Ltd, Renfrew, UK): primers AJ245 and AJ246 based on the genome *ams* region (Jones and Geider, 2001) and pEA29 A and pEA29 B based on plasmid pEA29 DNA (Bereswill *et al*., 1992). The amplification was carried out in a total volume of 25 μl containing (final concentrations) 1× PCR buffer (STS Ltd), 5 mM $MgCl_2$, 0.125 mM of each dNTP, 0.4 U *Taq* DNA polymerase (STS Ltd), 10 pmol of each primer, 1 μl of template DNA, under the following reaction conditions: a denaturation step at 94 °C for 4 min was followed by 35 cycles at 94 °C for 45 s, 58 °C for 45 s and 72 °C for 1 min. A final step at 72 °C for 5 min stopped the reaction. The PCR products were separated electrophoretically on 1.5% agarose gel in TBE buffer (Maniatis *et al*., 1982) (1 h at 100 V), stained with ethidium bromide, and photographed under UV light.

Antibiotic susceptibility

In vitro susceptibility towards 3 antibiotics was determined by the Kirby-Bauer technique (Bauer *et al*., 1966) using Oxoid sensitivity discs. Bacterial suspensions of about 10^9 cells/ml were prepared in physiological saline from 24-h-old King's B cultures and swabbed on King's B plates. After incubation at 28 °C for 24 h the diameter of the inhibition zones was measured.

SDS-PAGE of cellular proteins

The strains were cultivated in 100 ml LB medium in Erlenmeyer flasks and aerated by rotary shaking (150 rpm) at 26 °C. The bacterial suspensions were standardized after measurement of their optical density at 600 nm. The cells were separated by centrifugation (8000 rpm for 5 min), and then twice washed with distilled water. The protease inhibitor phenyl-methane-sulfonyl-fluoride (Sigma Chemical Co., St. Louis, USA) was added to the final concentration of 1 mM. The samples were frozen at −70 °C until used for further analyses. 1 ml of the defrosted bacterial cells was resuspended in 0.5 ml 2× SDS lytic buffer [0.125 M Tris [tri(hydroxymethyl)-aminomethane]-hydrochloride, pH 6.8, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol] mixed well by vortexing, incubated for 4 min at 100 °C, and then centrifuged (11752 × *g* for 20 min) in order to remove any remaining whole cells or their fragments, and to obtain the cell-free extracts.

SDS-PAGE was performed by the method of Laemmli (1970). The proteins were analyzed on 1.5 mm thick and 160 mm long gels run in the dual vertical slab unit Hoefer SE 400 (Amersham Pharmaci Biotech). From each sample, 10 μl aliquots were loaded on a polyacrylamide gel. The following low-molecular protein standards (Amersham Biosciences) were used: α-lactalbumin (14.4 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (30 kDa), ovalbumin (45 kDa), albumin (66 kDa), phosphorylase B (97 kDa). Electrophoresis was carried out at a constant current of 35 mA through the stacking (4%) and the separation gel (15%) at room temperature.

Results and Discussion

Isolation and initial characterization

The strains included in this study originated from infected plant material and were obtained as distinct colonies with characteristic morphology of *E. amylovora* on King's medium B. The colonies that were oxidase-negative, catalase-positive and consisted of Gram-negative motile rods were picked after cultivation at 26 °C for 24–48 h. After the purification procedures and screening for fermentation of glucose with gas production and nitrate reduction, fifty-three strains were selected. All the selected strains fermented glucose without gas formation and did not reduce nitrate. The strains did not grow at 36–39 °C and in the presence of 5% NaCl. Plated on sucrose nutrient agar they formed mainly one morphological type of colonies – white, domed, shiny, mucoid with radial striations and a dense, flocculent centre (Billing *et al*., 1961). They formed the typical yellow, circular and smooth colonies on MM2Cu medium (Bereswill *et al*., 1997). The strains did not show a mucoid growth on YDC medium. All the isolates possessed the morphological properties characteristic for *E. amylovora* (Holt *et al*., 1994; Jones and Geider, 2001).

Identification

API 20E and BIOLOG systems

The API 20E system showed that all the strains possessed the biochemical properties (Table II) described for *E. amylovora* (Holt *et al*., 1994; Jones and Geider, 2001). The tests of the system BIO-

Table II. Metabolic diversity of *E. amylovora* isolates determined by the API 20E system.

API 20E profile	Number of the strains from different host plants										Total number
	Pyrus	*Malus*	*F. moshata*	*F. ananassa*	*Cydonia*	*C. integerrimus*	*P. coccinea*	*Crataegus*	*A. melanocarpa*		
0007562	8 (1, 4, 10, 18, 19, 20, 34, *Ea**)	3 (51, 52, 236)			1 (31)				**1** (15)		13
0007772	2 (1C, 2C)		1 (247)	2 (244, 245)	4 (22, 23, 24, 29)	1 (28)	3 (25, 26, 27)				13
0007162	3 (3, 5, 13)		1 (237)								4
0007062	1 (7)	3 (12, 39, 40)									4
0007462	3 (9, 16, 21)	1 (42)									4
0007060	1 (17)	2 (49, 55)									3
0005060	2 (33, 34)										2
0006565	2 (2, 32)										2
0007522	2 (6, 8)										2
0007762								**1** (36)	**1** (14)		2
0007022		1 (11)									1
1007562	1 (30)										1
1007772	1 (246)										1
0006062		1 (54)									1
0007572			1 (238)								1
Total number	26	11	3	2	5	1	3	1	2		54

* *Ea*, Type strain of *E. amylovora* ATCC 15580. The designation of the strains in the brackets corresponds to that in Table I.

LOG identified all the strains as *E. amylovora* at a similarity level between 0.746 and 0.962.

PCR amplification

The belonging of the isolates to the species of *E. amylovora* was confirmed by PCR amplification of the genome *ams* region and the specific region of plasmid pEA29. *E. amylovora* ATCC 15580 and related *E. pyrifoliae* DSM 12163 were used as controls. All the isolates were subjected to PCR amplification using the primers AJ245 and AJ246, so that the presence of the ams chromosome region specific for *E. amylovora* could be proved. All the isolates displayed a positive reaction for the presence of the *ams* region, the same as the type culture of *E. amylovora* (Fig. 1). The amplified product was about 519 bp in all strains and corresponded to preliminary sequential analysis of GenBank data. *E. pyrifoliae* gave also a positive reaction. This was due to the substantial homology between the *ams* genes and the genes coding for capsular exopolysaccharide (structurally similar to amylovoran), which is required for the pathogenicity of *E. pyrifoliae* (Jock *et al*., 2003). The results obtained showed that the use of this pair of primers alone was not sufficient to confirm the species identification of the strains as *E. amylovora* because of the cross-reaction in the closely related species *E. pyrifoliae*.

To avoid the disadvantage of this pair of primers and to confirm the species identification of the strains, we performed PCR amplification of a specific region of plasmid pEA29 of *E. amylovora*. It is known that all *E. amylovora* strains possess a low copy number plasmid pEA29 (Falkenstein *et al*., 1989; Laurent *et al*., 1989). On this basis, Bereswill *et al*. (1992) proposed for the detection of *E. amylovora* by PCR the primers pEA29 A and pEA29 B, specific to a DNA fragment of pEA29, which we used in this study. All the strains, but not *E. pyrifoliae*, gave, after PCR, a single DNA fragment of a length between 1000 and 1100 bp (Fig. 2). Certain polymorphism was observed in the lengths of the amplified fragments among the strains, which confirmed the results of Bereswill *et al*. (1993) and McManus and Jones (1995).

Pathogenicity tests

All isolates provoked a hypersensitive reaction on tobacco leaves observed 18–24 h after infiltration, and produced the typical symptoms of pear twigs.

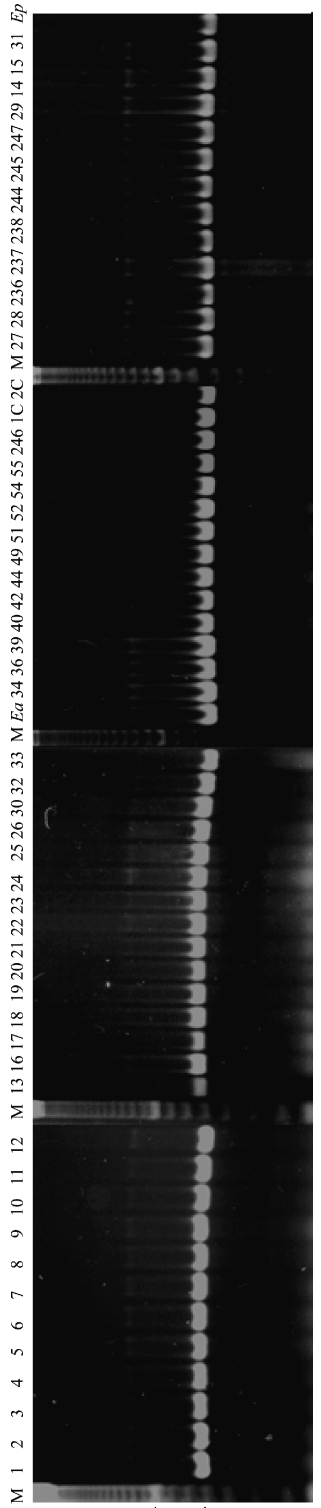

Fig. 1. PCR amplification with two primers (AJ245 and AJ246) of Bulgarian *E. amylovora* strains isolated from different host plants. The number of each lane corresponds to the designation of the strain analyzed. *Ea*, type strain of *E. amylovora* ATCC 15580; *Ep*, type strain of *E. pyrifoliae* DSM 12163; M, 100 bp DNA marker (Amersham Biosciences, Vienna, Austria).

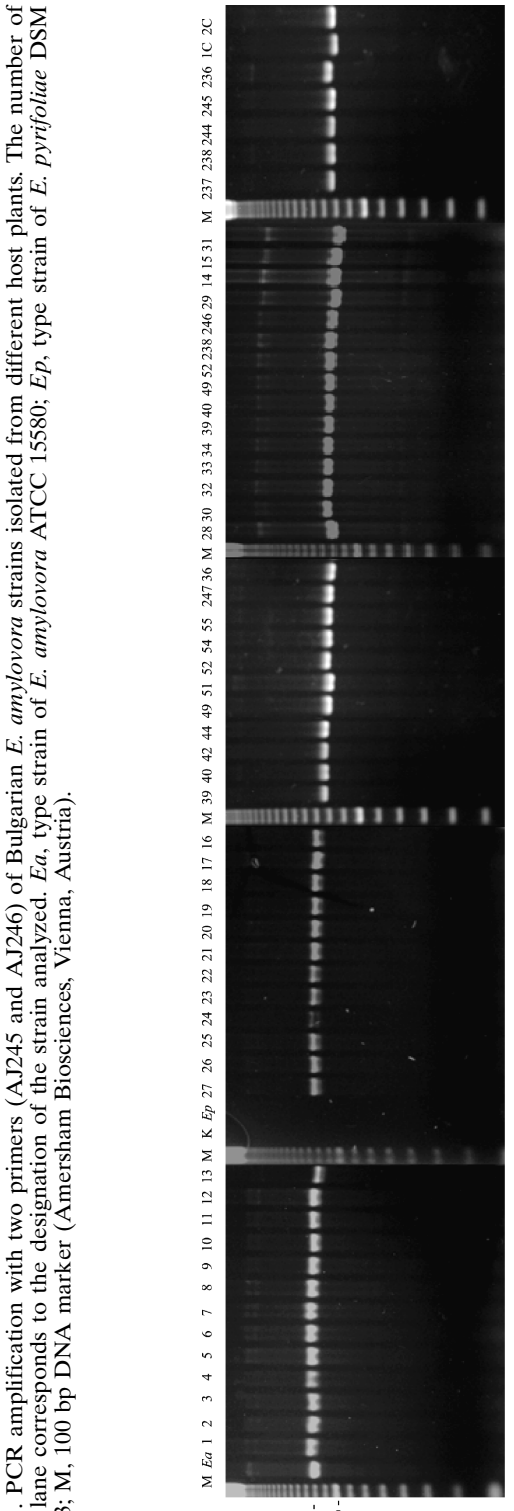

Fig. 2. PCR amplification of Bulgarian *E. amylovora* strains isolated from different host plants with the primers pEA29 A and pEA29 B. The number of each lane corresponds to the designation of the strain analyzed. *Ea*, type strain of *E. amylovora* ATCC 15580; *Ep*, type strain of *E. pyrifoliae* DSM 12163; M, 100 bp DNA marker (Amersham Biosciences, Vienna, Austria).

Phenotypic diversity

The data obtained by the systems API 20E and BIOLOG were used to assess the metabolic diversity among the strains. A characteristic API 20E profile was obtained for each strain. The strains formed two major and 13 minor groups on the basis of their API 20E profile. The dominant profiles were 0007562 and 0007772 (Table II). The comparison of our results with those of Mergaert *et al.* (1984), Vantomme *et al.* (1986) and Donat *et al.* (2005), who characterized *E. amylovora* strains by the same system, disclosed that our strains differed in their API 20E profiles. Only three pear isolates possessed the already reported API 20E profiles (0007522 and 0007022). The profiles of the majority of the strains were not registered in previous studies.

The comparison of the BIOLOG profiles of the strains showed that 41 tests out of 95 carbon sources tests got differential responses (Table III). The

Table III. Biolog GN substrates differentially used by the strains and associated to *E. amylovora* groups defined by 70% similarity.

Substrate	Group				
	A	B	C	D	E
Dextrin	92	–	–	–	–
N-Acetyl-D-glucosamine	92	+	+	+	+
Adonitol	–	29	–	54	–
D-Cellobiose	–	–	–	27	–
L-Fucose	–	–	–	18	–
D-Galactose	+	+	+	+	40
Gentiobiose	+	+	+	+	40
m-Inositol	58	35	+	+	–
D-Mannitol	+	+	+	+	80
D-Mannose	8	–	–	9.1	–
D-Psicose	92	18	–	18	–
D-Raffinose	58	12	+	54	50
Turanose	17	12	–	–	–
Pyruvic acid methyl ester	+	–	+	18	–
Succinic acid monomethyl ester	75	23	–	82	–
Acetic acid	–	–	–	9	–
D-Galacturonic acid	–	12	–	27	–
D-Gluconic acid	+	94.1	+	+	40
D-Glucoronic acid	–	–	–	9.1	–
α-Hydroxybutyric acid	8	–	–	–	–
α-Ketoglutaric acid	92	6	–	45	–
D,L-Lactic acid	75	–	–	9	–
Succinic acid	92	76	+	82	–
Bromosuccinic acid	+	+	+	64	–
Succinamic acid	50	35	–	9.1	–
L-Alaninamide	42	–	–	–	–
L-Alanine	67	–	–	–	–
L-Alanyl-glycine	42	–	–	–	–
L-Asparagine	50	41	–	27	–
L-Aspartic acid	+	94	+	+	–
L-Glutamic acid	+	94	+	+	–
Glycyl-L-glutamic acid	58	47	–	54	–
L-Histidine	17	6	–	27	–
L-Proline	67	–	–	9	–
L-Serine	25	–	–	18	–
Urocanic acid	–	–	+	45	–
Inosine	+	94	+	+	–
Uridine	+	82	+	45	–
Thymidine	33	88	+	54	–
Glycerol	+	88	+	18	10
D,L-α-Glycerolphosphate	–	–	+	9	–

+, all the strains in the group were positive; –, all the strains in the group were negative; number, percentage of positive strains in the group.

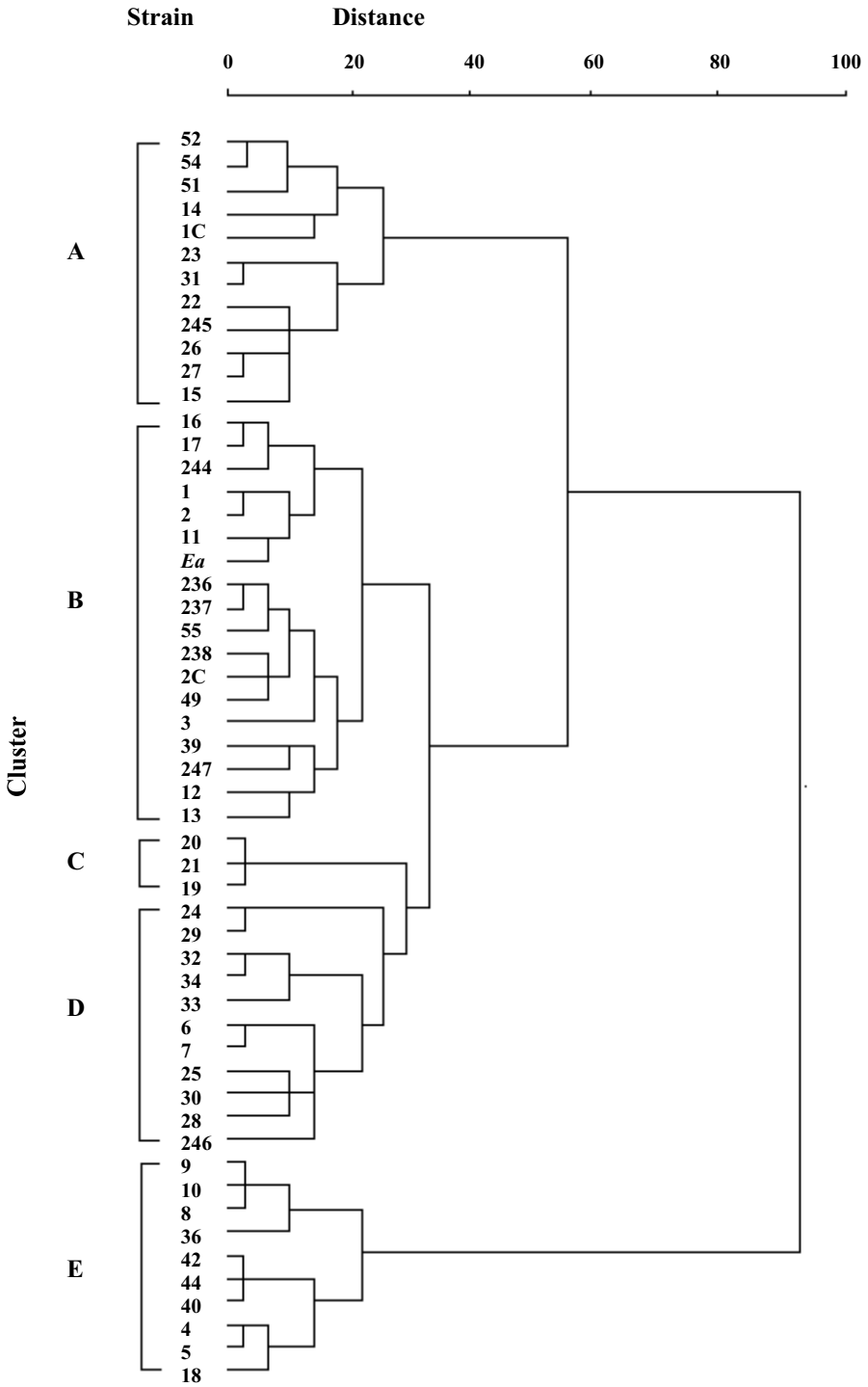

Fig. 3. Dendrogram showing BIOLOG clusters of 53 *E. amylovora* strains, obtained by SPSS. The numerals correspond to the designation of the strains analyzed. *Ea*, type culture of *E. amylovora* ATCC 15580.

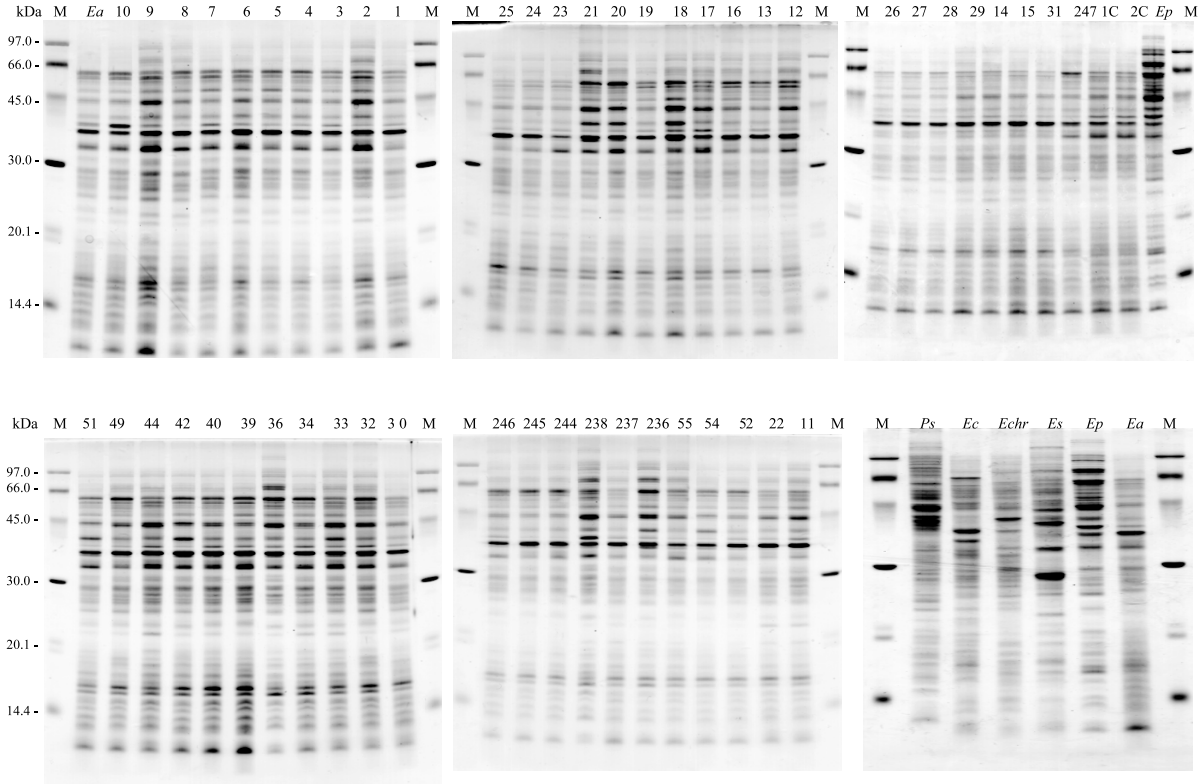

Fig. 4. SDS-PAGE analysis of the strains. The number of each lane corresponds to the designation of the strain analyzed. *Echr*, type culture of *E. chrysanthemi* ATCC 11663; *Es*, type culture of *E. stewartii* ATCC 8199; *Ps*, type culture of *P. syringae* pv. *syringae* NBIMCC 2420; *Ec*, type culture of *E. coli* ATCC 10536; *Ea*, type culture of *E. amylovora* ATCC 15580; *Ep*, type strain of *E. pyrifoliae* DSM 12163.

remaining 54 tests were either positive or negative for all the strains. The results from the BIOLOG plates for each *E. amylovora* strain were subjected to cluster analysis. The matrix of similarity between the isolates was calculated using the Euclidian distance. Cluster analysis was performed by the Ward method through the cluster analysis procedure of SPSS. This analysis defined 5 groups at 70% similarity (Fig. 3). Group A consisted of 12 strains isolated from 6 host plants (*Malus* sp., *Cydonia* sp., *Aronia melanocarpa*, *Pyracantha coccinea*, *Pyrus* sp., and *Fragaria ananassa*). All the strains of the group did not utilize 54 of the substrates, assimilated 18 of them, and showed different reaction to the remaining 23 substrates. The major group B included 17 isolates from four host plants (*Pyrus* sp., *Malus* sp., *Fragaria moshata*, and *F. ananassa*), as well as the type strains of *E. amylovora*. The strains of this group did not assimilate 61 of the substrates tested, they all utilized 12 of the substrates and differed in the reaction to 22 of them. Group C clustered 3 strains isolated from *Pyrus* sp., which did not differ between themselves. These strains did not utilize 70 and assimilated 25 of the substrates. Group D consisted of 11 strains from four host plants (*Pyrus* sp., *Cydonia* sp., *P. coccinea*, *Cotoneaster integerrimus*). All they did not assimilate 52 of the compounds and possessed the ability to utilize 18 of them. The strains differed in the reaction to 24 of the substrates of the system. Group E (10 strains from three host plants – *Malus* sp., *Pyrus* sp., and *Crataegus* sp.) was formed on the basis of low assimilation capacity of the strains included. The common characteristic of these strains is the inability to utilize 80 of the compounds and the assimilation of nine of them. They showed a different reaction to the remaining 6 substrates. Although the relationship between the groups formed and the strain origin did not reveal, the metabolic diversity of the

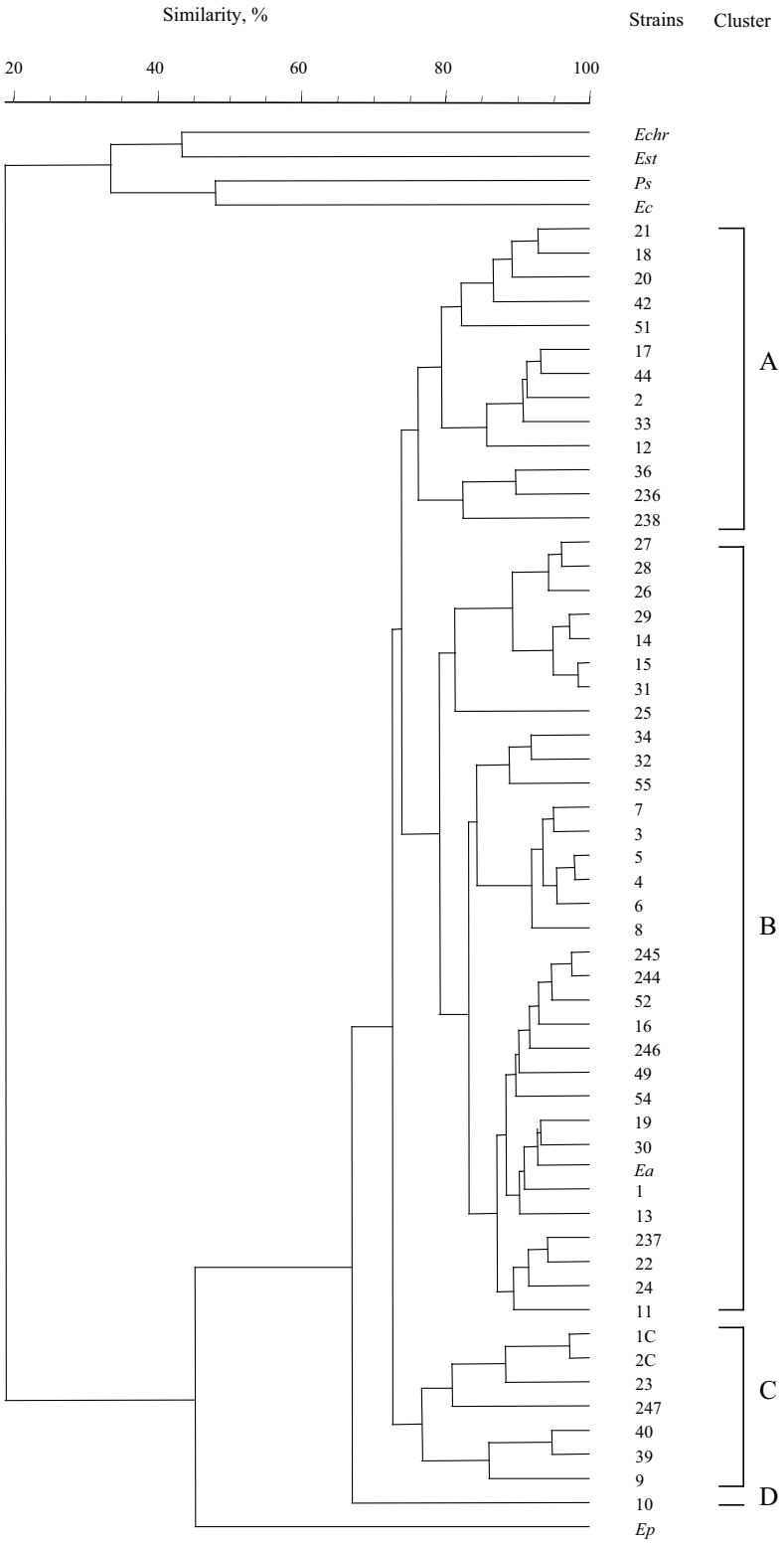

Fig. 5. Dendrogram showing SDS-PAGE clusters of 53 *E. amylovora* strains, obtained by UPGMA. The number of each lane corresponds to the designation of the strain analyzed. *Echr*, type culture of *E. chrysanthemi* ATCC 11663; *Est*, type culture of *E. stewartii* ATCC 8199; *Ps*, type culture of *P. syringae* pv. *syringae* NBIMCC 2420; *Ec*, type culture of *E. coli* ATCC 10536; *Ea*, type culture of *E. amylovora* ATCC 15580; *Ep*, type strain of *E. pyrifoliae* DSM 12163.

Table IV. Susceptibility of Bulgarian strains of *E. amylovora* to antibiotics.

Antibiotic	Number of strains		
	Resistant[a]	Intermediate susceptible[b]	Strong susceptible[c]
Streptomycin	0	52	1
Tetracycline	0	29	24
Oxytetracycline	0	20	33

[a] Inhibition zone ≤ 12 mm.
[b] Inhibition zone between 13 and 20 mm.
[c] Inhibition zone more than 20 mm.

strains studied was evident. Our findings confirmed the results obtained by Olamendi (2005), which determined 11 different metabolic BIOLOG profiles, but did not establish a connection with the origin of the strains.

Protein electrophoregrams were prepared from the 53 isolates of *E. amylovora*. The type cultures of *E. amylovora* ATCC 15580, *Erwinia stewartii* ATCC 8199, *Erwinia chrysanthemi* ATCC 11663, *E. pyrifoliae* DSM 12163, *Pseudomonas syringae* pv. *syringae* NBIMCC 2420, and *Escherichia coli* ATCC 10536 were used as control (Fig. 4).

All the investigated strains of *E. amylovora* displayed very similar protein profiles. The SDS-PAGE analysis of total soluble cellular proteins revealed at least 20 detectable bands in each strain (data not shown). The results were subjected to a cluster analysis, which was performed on the matrix of correlation values by UPGMA. At 75% similarity four groups were formed (Fig. 5). The major one (group B) included 62% of the strains, as well as the type culture of *E. amylovora.* That disclosed the great homogeneity of the populations of *E. amylovora*. Groups A and C contained 24% and 13% of the strains, respectively. Strain 10 alone differed in its protein pattern and formed a separate group. The analysis of the data showed that each group included the strains from different host plants and no relation between their origin and the protein profiles could be revealed. Zarnowski *et al*. (2002) studied the diversity between four *E. amylovora strains* of different origin and showed a high level of similarity on the basis of SDS-PAGE analysis, but discrete differences in the relative content of a single protein were also revealed. Small differences in the protein patterns among the Bulgarian isolates could be found in the area between 30 and 97 kDa. In most of the cases, the differences were quantitative. The two Serbian strains were in one cluster with a small number of Bulgarian isolates. This might be due to a common initial origin. Despite the fair relative homogeneity of the strains, the differentiation of four groups at 75% similarity indicated for a relative diversity in the protein profiles. The results obtained proved also the aplicability of this analysis for the identification of *E. amylovora.* It was shown (Fig. 5) that the species possessed a very characteristic protein profile, which differed greatly from those of *E. chrysanthemi, E. pyrifoliae, E. stewartii*, as well as *E. coli* and *P. syringae.*

The susceptibility of all the isolates to three antibiotics was established. These antibiotics were selected based on their application for fire blight control in several countries (Saygili and Üstün, 1996; Psallidas and Tsiantos, 2000). The diameter of the inhibition zones after incubation of the strains on King's B medium with antibiotic discs was recorded. The strains were distributed into three groups (Table IV) according to the size of the inhibition zones – resistant (inhibition zone 12 mm or less), intermediate susceptible (inhibition zone 13–20 mm), and strongly susceptible (inhibition zone more than 20). None of the Bulgarian strains tested showed resistance to streptomycin, tetracycline and oxytetracycline. The results obtained in this study showed that the *E. amylovora* populations in Bulgaria were sensitive to the antibiotics tested, which could be explained by the initial origin of the Bulgarian strains from the countries where the use of these antibiotics is forbidden.

The isolation and identification of fifty-one strains from different host plants made it possible to conclude that *E. amylovora* is widely spread in Bulgaria. The phenotypic diversity evaluation of the *E. amylovora* strains indicated that the Bulgarian strains varied metabolically among themselves,

as well as with the others reported by several authors. No connection between the origin of the strains and metabolic diversity was revealed.

Acknowledgements

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