OCCURRENCE OF PHYTOPATHOGENIC BACTERIA OF *ENTEROBACTERIACEAE* FAMILY IN BULBS OF CULTURAL AND ORNAMENTAL PLANTS

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ABSTRACT

This paper presents an analysis of the phytopathogenic bacteria with facultative anaerobic growth found in diseased bulbs of onion, garlic, gladiolus, tulip, hyacinth, narcissus, and lily in the period 2003-2011. The isolated strains belong to *Pantoea agglomerans, Pectobacterium carotovorum, Ewingella americana, Rahnella aquatilis, Serratia* sp. and *Enterobacter* sp. and cause typical symptoms of bulb rot under natural conditions and after artificial contamination.

Key words: *phytopathogenic bacteria, Enterobacteriaceae, bulbs*

INTRODUCTION

The *Enterobacteriaceae* is a large family of Gram-negative mesophilic bacteria of worldwide distribution. They are facultative anaerobes with relatively simple growth requirements, capable of fermenting a variety of different carbohydrates to produce various end products [1,9] The patterns of this fermentation are used for species differentiation [9].

Although *Enterobacteriaceae* are commonly known as a part of the normal intestinal tract flora, these bacteria are found in a diverse variety of environments occurring as saprophytes or parasites in plants and animals [1,8]. Interactions with host species may be benign, mutualistic or pathogenic. Plants are the primary hosts for many members of *Enterobacteriaceae* family, mainly from genera *Erwinia, Pectobacterium, Dickeya, Pantoea, Enterobacter,* and *Brenneria*, which behave as epiphytes, endophytes and/or pathogens [8] However, animal pathogenic enterobacteria are also able to colonize plants and even be opportunistic phytopathogens [8,20].

Several members of the *Enterobacteriaceae* family are known to cause diseases of the underground parts of plants. *Enterobacter cloacae* infects onion bulbs. Bulb decay symptoms are generally absent in the field, but appear on the internal scales after one to three months in storage [4]. *Erwinia rhapontici* infects several plant hosts and causes „internal browning" of hyacinth and rot of onion bulbs damaging the internal scales [5,17]. *Pantoea agglomerans* is generally known as a saprophyte but the pathogenic strains cause stem necrosis of onion and garlic, and bulb rot of onion [7,12]. *Pantoea ananatis* also infects onion with symptoms of leaf blight and center rot of bulbs [16]. Phytopathogenic strains of the soil- and wáter-borne bacteria *Serratia marcescens*, also known as a human pathogen, were found. They were reported to cause decay of stored onion bulbs [6]. The most typical pathogen of bulb-forming plants is *Pectobacterium carotovorum*. This polyphagous bacteria synthesizes pectolytic enzymes causing the well-known disease 'soft rot' of more than 200 plants including the overground parts and the bulbs of different *Allium* species, gladiolus, tulip, hyacinth, narcissus, and lily [5]. More rarely, the causal agent of soft rot of onions, hyacinth, and tulip can be *Dickeya chrysanthemi* [15]. Characteristic symptoms of the disease are watery, sticky tissues and strong unpleasant smell.

This paper presents an analysis of the phytopathogenic bacteria with facultative anaerobic growth from the *Enterobacteriaceae* family found in diseased bulbs of onion, garlic, gladiolus, tulip, hyacinth, narcissus, and lily in the period 2003-2011 in Bulgaria.

MATERIALS AND METHODS

Bacterial strains were recovered from diseased bulbs of onion, garlic, gladiolus, tulip, hyacinth, narcissus, and lily collected in the period 2003-2011 from the market in Bulgaria. The

bulbs originated from Bulgarian growing fields and greenhouses and three of them had been from imported consignments. The bulbs had watery soft internal scales with yellow to brown tissues. Bacteria were isolated on King's medium B. The pathogenic properties of the isolates were confirmed by infiltration of tobacco cv. Samsun NN [11] and by an artificial inoculation of onion bulb scales and whole onion bulbs [19]. The presence of hypersensitive reaction (HR) was observed between 18-24 hours after inoculation and the symptoms of the disease were examined periodically between 1^{st}-5^{th} day.

For the purpose of the study screening for the major *Enterobacteriaceae* characteristic properties was performed: Gram-reaction, anaerobic growth, oxidase activity. Species identification of the isolates was carried out by the Biolog system in GN2 MicroPlates and data analysis with the MicroLog 4.20.05 (Biolog™). For identification purposes API 20E strips for some of the strains were also used. The 8-digid code was compared with the API 20E v4.0 database. Metabolic patterns were further processed by SPSS16 hierarchical clustering based on the Ward's method.

Nine strains were subjected to RFLP analysis as described by [13]. DNA was extracted from bacterial cultures grown in Luria-Bertrani Broth at 28°C, 200 rpm, overnight by DNeasy Blood&Tissue Purification Kit (QIAGEN) according to the manufacturer's instructions. The PCR was performed with eubacterial primer pair U1 (5'CCAGCAGCCGCGGTAATACG3') and U2 (5'ATCGG(C/T)TACCTTGTTACGACTTC3'). PCR mix contained 1x buffer, 2.5mM $MgCl_2$, 50*p*mol each primer, 0.02mM dNTPs, and 1.25U *Taq*-polymerase final concentrations. 100ng of DNA to each reaction tube was added. Amplification was performed with initial denaturation at 94°C for 10 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, and a final elongation at 72°C for 10 min [13]. PCR products were separated in 1% agarose gel in 1x TBE buffer at 100V for 1h. Endonucleases *Bsp*119I and *Dde*I (Fermentas) were used for digestion of the PCR product. The reaction mixtures (25µl) contained 8µl PCR product, 1µl enzyme (10 U), and 2.5µl restriction buffer, and were incubated at 37°C for 2h. Digested products were separated in 2% agarose gel in 1x TBE buffer at 100 V for 1h.

RESULTS AND DISCUSSION

A total of 40 representative pathogenic strains of the *Enterobacteriaceae* family (Gram-negative, oxidase-negative, facultative anaerobs) for the 8-year period were investigated (Tabl. 1).

Tabl. 1. Pathogenicity and origin of the isolates

Strains	Year	Origin	Host	Pathogenic	HR
1E, 8E	2003	BG	onion	yes	yes
17E, 18E	2004	BG	onion	yes	yes
19E, 48E	2004	BG	onion	yes	no
23E, 24E	2004	imported	onion	yes	no
49E, 50E	2004	BG	onion	no/colonization	no
30E	2004	imported	garlic	yes	yes
33E	2004	BG	garlic	yes	yes
31E, 32E	2004	BG	garlic	yes	no
70E, 84E	2004	BG	gladiolus	yes	yes
74E	2004	BG	gladiolus	yes	no
53E, 56E	2005	BG	onion	yes	yes
52E, 54E, 61E, 63E	2005	BG	onion	yes	no
89E	2005	BG	lily	yes	yes
98E	2005	BG	gladiolus	yes	yes
130E	2006	BG	onion	yes	yes
100E	2006	BG	narcissus	yes	yes
116E, 117E, 123E	2006	BG	tulip	yes	yes
106E	2006	BG	hyacinth	yes	yes
101E	2006	BG	hyacinth	yes	no
52 la	2008	BG	tulip	yes	no
11 la, 10 la	2009	imported	tulip	yes	no
1(1)l, 1(3)l, R2(5)l	2011	BG	tulip	yes	yes
4(3)n, R6(10)n	2011	BG	narcissus	yes	no

The strains induced pathological changes in plant tissues upon artificial inoculation. Nineteen of the strains caused symptoms of disease up to the 3^{rd} day after inoculation of onion bulb scales and nineteen of the strains - between 3^{th}-6^{th} day. The infected scales became watery, soft, with beige or yellow-beige darkening to beige color. Two strains induced development of beige-reddish color which turned to beige. Two weeks after inoculation of whole onion bulbs the internal bulb scales showed similar symptoms of soft beige scales. Some of the bulbs did not germinate and those that germinated showed deformed leaves. In a month the leaves developed chlorosis and finally the whole plants died opposed to the healthy control plants (Fig. 1). Two of the strains successfully colonized the bulb

scales forming abundant exudates but pathological changes in plant tissues were not observed.

Twenty-five of the strains induced hypersensitive reaction in tobacco leaves up to 24 hours after inoculation (Tabl. 1). The hypersensitive reaction activity did not show relation to the speed of appearance of the symptoms of disease of onion bulb scales.

The bacterial isolates belonging to the *Enterobacteriaceae* family formed mostly mucous even-ended raised colonies whitish or yellow-pigmented on King's B agar medium. They shared common reaction to 20 of the substrates included in Biolog plates. The strains utilized β-D-glucose, D-fructose, m-inositol, D-mannitol, D-mannose, b-methyl-D-glucoside, glycerol, L-aspartic acid,

Fig. 1. Symptoms of disease after artificial inoculation of onion scales and onion bulbs. The infected bulbs did not germinate or germinated with weak chlorotic leaves followed by dieback of the whole plant

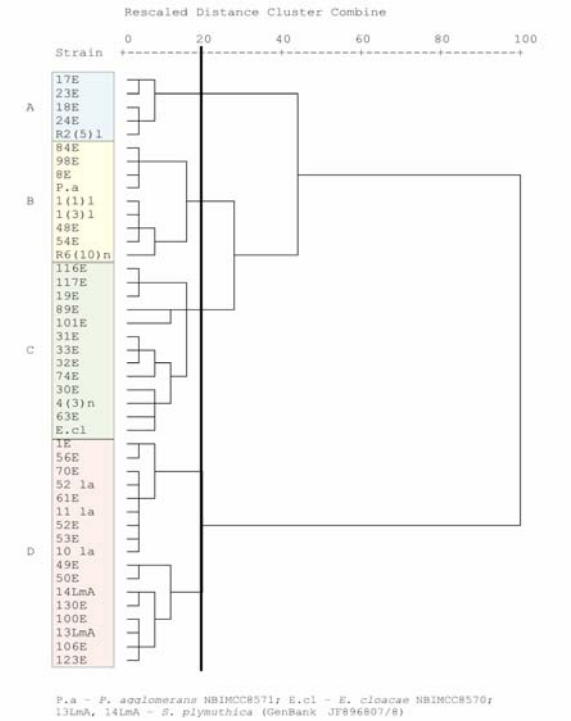

Fig. 2. Hierachical cluster analysis of the strains based on Biolog metabolic data

D-glucose-1-phosphate, and D-glucose-6-phosphate, and did not utilize itaconic acid, α-keto butyric acid, α-keto valeric acid, sebacic acid, succinamic acid, L-leucine, D-L-carnitine, L-pyroglutamic acid, phenylethylamine, and 2,3-butanediol. The data obtained by the Biolog system was used to assess the interspecies metabolic diversity. It was subjected to cluster analyses which defined 4 groups at 80% similarity (Fig. 2).

Cluster A is homogeneous, composed of 5 strains with similar characteristics and more than 90% similarity (Fig. 2). They utilized 35 carbon sources, did not utilize 40 substrates, and differed in their reaction to 18 carbon sources. Weak reaction was observed towards 2 substrates (Tabl. 2). The strains were identified as *Ewingella americana* by MicroLog with probability (PROB) 100%, similarity index (SIM) 0.594-0.800, and distance index (DIST) 3.04-6.32. A characteristic feature of these strains seemed to be the lack assimilation of L-arabinose which was utilized by all other isolates from the *Enterobacteriaceae* family.

Group B consists of strains identified by MicroLog as *P. agglomerans* with PROB 94-100%, SIM 0.544-0.940, and DIST 0.920-7.120. This cluster unites all *P. agglomerans* with the exception of one strain which stands in cluster C. They utilized 26 carbon sources, did not utilize 24 substrates, and differed in their reaction to 45 carbon sources (Tabl. 2).

Cluster C comprises unidentified and identified strains biochemically close to *E. cloacae* (Fig. 2). The bacteria in that group shared their reaction to 38 utilizing 19 carbon sources (Tabl. 2). Five of the strains were standing closest to or identified as *Rahnella aquatilis* with PROB 83-95%, SIM 0.530-0.630, and DIST 5.090-5.630. One strain was identified as *Pectobacterium carotovorum* (with PROB 99%, SIM 0.750, and DIST 3.680) although the symptoms of disease of plant tissues induced by that isolate lacked the strong unpleasant odor typical for the species. One strain was supposed to be *E. cloacae* with PROB 100%, SIM 0.670, and DIST 5.060 and four strains behaved metabolically as *Enterobacter* sp.

Table 2. Biolog substrates differentially used by the groups and the identified species.

Substrate	Cluster				Species				
	A	B	C	D	*E.americana*	*P.agglomerans*	*R.aquatilis*	*K.oxytoca*	*Serratia* sp.
Cyclodextrin	-	-	(8.3)	(73.33)	-	-	-	(+)	v
Dextrin	80	77.5(12.5)	83.3(8.3)	+	v	v	v	+	+
Glycogen	-	(12.5)	(33.3)	53.3(46.7)	-	v	v	+	v
Tween 40	20(50)	25(50)	16.7(50)	93.4(6.6)	v	v	v	+	v
Tween 80	20(50)	37.5(62.5)	16.7(58.3)	+	v	v	v	+	+
N-Acetyl-D-Galactosamine	+	25	8.3(8.3)	73.33(6.6)	+	v	v	+	+
N-Acetyl-D-Glucosamine	+	77.5	+	+	+	v	+		+
Adonitol	-	-	7.7(7.7)	46.7(33.3)	-	-	-	+	v
L-Arabinose	-	+	+	+	-	+	+	+	+
D-Arabitol	+	62.5	25(8.3)	60(40)	+	v	v	+	v
D-Cellobiose	+	50(12.5)	91.7(8.3)	93.4(6.6)	+	v	v	+	v
I-Erythritol	-	12.5	16.7	(33.3)	-	v	v	-	v
D-Fucose	+	12.5	33.3(8.3)	+	+	v	v	+	+
D-Galactose	+	77.5(12.5)	+	+	+	v	+	+	+
Gentibiose	+	37.5(12.5)	+	93.4(6.6)	+	v	+	+	v
α-D-Lactose	+	50	66.6(16.7)	86.7	+	v	+	+	v
Lactulose	+	-	58.3(8.3)	93.4	+	-	+	+	v
Maltose	+	+	91.7	+	+	+	+	+	+
D-Melibiose	-	(25)	83.3(16.7)	+	-	v	+	+	+
D-Psicose	+	37.5(37.5)	33.3(58.3)	+	+	v	v	+	+
D-Raffinose	-	37.5(25)	83.3(16.7)	+	-	v	+	+	+
L-Rhamnose	20	+	+	60(26.6)	v	+	+	+	v
D-Sorbitol	-	12.5(12.5)	66.7(8.3)	+	-	v	v	+	+
Sucrose	-	77.5	+	+	-	v	+	+	+
D-Trehalose	+	+	91.7	+	+	+	v	+	+
Turanose	-	(25)	8.3(33.3)	+	-	v	v	+	+
Xylitol	-	-	16.7	40(53.3)	-	-	v	+	v
Pyruvic Acid Methyl Ester	40(60)	+	83.3(16.7)	+	v	+	v	+	+
Succinic Mono-Methyl Ester	(+)	50(50)	41.7(33.3)	+	(+)	v	v	+	+
Acetic Acid	(60)	62.5	66.7	66.7(33.3)	v	v	(+)	v	v
Cis-Aconitic Acid	80(20)	77.5(12.5)	58.3(8.3)	+	v	v	v	+	+
Citric Acid	+	62.5(12.5)	75(8.3)	+	+	v	v	+	+
Formic Acid	(+)	(50)	8.3(50)	73.3(33.3)	(+)	v	v	+	v
D-Galactonic Acid Lactone	+	12.5	50	6.6(46.6)	+	v	-	v	v
D-Galacturonic Acid	+	77.5(12.5)	+	+	+	v	+	+	+
D-Glucoronic Acid	+	+	91.7	+	+	+	+	+	+
Glucosaminic Acid	+	12.5(12.5)	33.3	73.3(13.3)	+	v	v	+	v
D-Glucuronic Acid	+	+	66.7(8.3)	+	+	+	v	+	+
α-Hydroxybutiric Acid	-	(12.5)	-	-	-	v	-	-	-
β-Hydroxybutiric Acid	-	-	-	6.6(26.6)	-	-	-	v	v
γ-Hydroxybutiric Acid	-	-	-	6.6(6.6)	-	-	-	v	v
p-Hydroxyphenylacetic Acid	-	-	-	+	-	-	-	+	+
α-Ketoglutaric Acid	-	(12.5)	8.3(16.7)	46.6(26.6)	-	v	v	(+)	v
D,L-Lactic Acid	20(40)	+	75(16.7)	+	v	+	v	+	+
Malonic Acid	-	(25)	16.7(8.3)	13.3	-	v	v	+	-
Propionic Acid	-	(12.5)	(8.3)	-	-	v	-	-	-
Quinic Acid	-	(50)	8.3(33.3)	40	-	v	v	+	v
Saccharic Acid	-	+	83.3	33.3	-	+	v	+	v
Succinic Acid	60(40)	+	91.7	86.7(6.6)	v	+	+	+	v
Bromosuccinic Acid	20(80)	77.5(12.5)	50(50)	+	v	v	v	+	+
Glucoronamide	-	75(25)	25(25)	(6.6)	-	v	v	v	-
L-Alaninamide	-	-	-	33.3(33.3)	-	-	-	(+)	v
D-Alanine	60(40)	75(25)	33.3(58.3)	+	v	v	v	+	+
L-Alanine	60(40)	+	83.3(8.3)	+	v	+	v	+	+
L-Alanyl-Glycine	60(40)	+	41.7(50)	+	v	+	v	+	+
L-Asparagine	80(20)	+	83.3(16.7)	+	v	+	v	+	+
L-Glutamic Acid	+	+	91.7	86.7	+	+	v	-	v
Glycyl-L-Aspartic Acid	40(60)	37.5(37.5)	-	46.6	v	v	-	+	v
Glycyl-L-Glutamic Acid	60(40)	25(62.5)	8.3(58.3)	33.3(33.3)	v	v	v	v	v
L-Histidine	20(80)	+	41.7(16.7)	+	v	+	v	+	+
Hydroxy-L-Proline	-	-	-	86.7	-	-	-	-	+
L-Ornithine	-	(62.5)	(25)	6.6(6.6)	-	v	v	-	v
L-Phenylalanine	-	-	(8.3)	-	-	-	v	-	-
L-Proline	+	+	41.7(25)	93.3	+	+	-	+	+
D-Serine	-	(12.5)	16.7(8.3)	86.7(13.3)	-	v	v	+	v
L-Serine	+	50	91.7	+	+	v	v	+	+
L-Threonine	(60)	-	8.3(8.3)	(13.3)	v	-	v	-	v
γ-Aminobutyric Acid	-	-	8.3(8.3)	26.6(13.3)	-	-	v	-	v
Urocanic Acid	-	25(12.5)	16.7(16.7)	86.7(6.6)	-	v	v	v	+
Inosine	+	62.5	+	+	+	v	+	+	+
Uridine	+	50(12.5)	66.7(16.7)	73.3(13.3)	+	v	v	+	v
Thymidine	+	62.5	66.7(25)	93.4(6.6)	+	v	v	+	v
Putrescine	-	-	-	93.4(6.6)	-	-	-	+	v
2-Aminoethanol	-	-	-	(26.6)	-	-	-	(+)	-
D,L-□-Glycerol Phosphate	+	62.5	+	+	+	v	+	+	+

+, positive; (+), weak positive; –, negative; v, variable; number, percentage of positive strains in the group; (number), percentage of weakly positive strains in the group.

Cluster D includes all strains belonging to genus *Serratia*. Two isolates identified as *Klebsiella oxytoca* and lacking pathogenic potential but having the ability to colonize plant tissues also locate here. The strains utilized 42 substrates, did not utilize 13, and differed in their reaction to 40 carbon sources (Tabl. 2). Four strains were identified as *Serratia* sp. by MicroLog and API, three were biochemically related to *Serratia* sp. and six strains remained unidentified with closest metabolic similarity to the *Enterobacter, Klebsiella,* and *Serratia* ID-candidates.

PCR for the tested strains gave the expected product of 996 bp. The RFLP analysis with two enzymes as described by Lu et al. [13] was implicated for some of the strains and the results were compared with the original paper (Fig. 3).

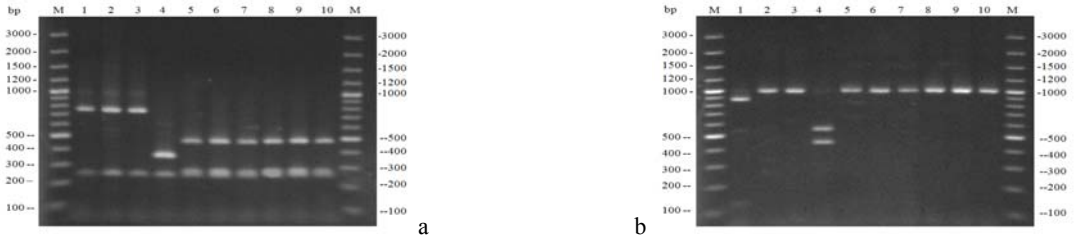

Fig. 3. RFLP profiles of some of the strains from clusters C and D with enzymes *Dde*I (a) and *Bsp*119I (b) (M-DNA ladder; 1-*E. cloacae*; 2-31E; 3-32E; 4-33E; 5-52E; 6-53E; 7-70E; 8-56E; 9-61E; 10-1E)

According to that method six strains behaved like *S. marcescens,* 32E identified by MicroLog as *E. cloacae* behaved in a different manner compared to *E. cloacae,* and 32E together with 31E gave products typical for *Escherichia coli*. As the original analysis was applied for clinical strains and did not include enough species from the *Enterobacteriaceae* family, we generally accept that six strains from Cluster D confirm their belonging to genus *Serratia* and that two strains from Cluster C belong to the *Enterobacter/Esherichia* group. The RFLP profile of strain 33E has not been described before.

The isolated phytopathogenic bacteria in this study belong to *P. agglomerans*, *P. carotovorum, E. americana, R. aquatilis, Serratia* sp. and *Enterobacter* sp. The identified species are distributed on plant hosts and through the years as shown on Fig. 4 and 5.

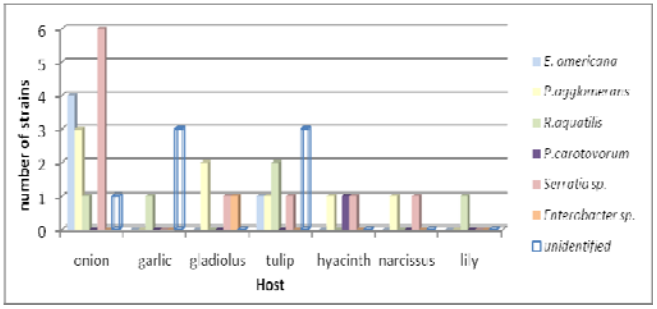

Fig. 4. Species of phytopathogenic *Enterobacteriaceae* distributed on plant hosts

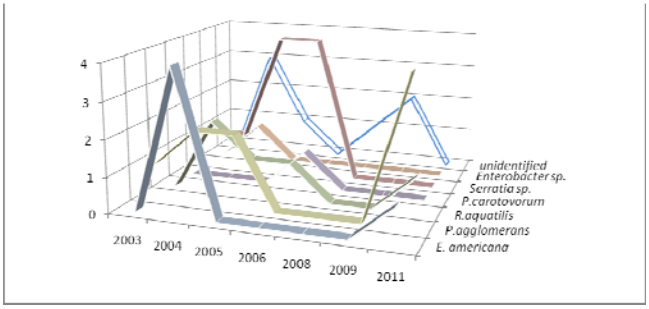

Fig. 5. Species of phytopathogenic *Enterobacteriaceae* distributed through the years

The isolated *Enterobacteriaceae* species found in onion, garlic, gladiolus, tulip, hyacinth, narcissus, and lily and cause typical symptoms of bulb rot under natural conditions and after artificial contamination. Diagnostics according to the symptoms of diseased plants could not be proposed due to the similar changes induces in plant tissues.

The identified pathogenic strains were recovered mostly from mono-morphological cultures in the isolation plates or together with non-pathogenic strains which were not further taken in consideration. In only two of the sample bulbs *Enterobacteriaceae* members occurred in mixed

infection with pathogenic bacteria from other taxonomic families: *R. aquatilis* was isolated together with *Pseudomonas fluorescens* from diseased garlic and *P. agglomerans* was isolated together with *Burkholderia gladioli* from gladiolus. All the strains from these samples induced symptoms in plant tissues after artificial inoculation and hypersensitive reaction in tobacco leaves. *Enterobacteriaceae* mixed infection was not observed.

The identified *Enterobacteriaceae* species proved to be common pathogens of bulbous plants that are able to cause infection without the presence of other pathogens. Symptoms of disease are initially located inside the bulb which makes diagnostics impossible. As members of *Enterobacteriaceae* family are capable of living in water and soil [3,6,10] it is possible that environment is the initial source of innoculum for the bulbs and that decayed bulbs maintain and enrich the infection in the soil. The identified bacteria with the exception of *E. americana* are known as phytopathogens of bulb-forming plants [4,5,6,7,12,18]. *E. americana* has been isolated from human and animals but its ecological niche remains unclear (Müller et al., 1995). However, it is a plant-associated bacterium in the rhizosphere [2].

In this study we found *P. agglomerans* as pathogen of onion, tulip, and narcissus, *Serratia* sp. – of onion, tulip, hyacinth, and narcissus, and *Enterobacter* sp. – of gladiolus for the first time in Bulgaria. Onion, garlic, tulip, and lily are reported as new hosts of *R. aquatilis*. To our knowledge this study is the first report of phytopathogenic strains of *E. americana* in onion and tulip.

REFERENCES

1. Anderson D., Novak P., Keith J., Elliott M. (eds.). 2007. Dorland's Medical Dictionary for Health Consumers. Saunders, Elsevier, Inc.

2. Babalola O., Akindolire A. 2011. Identification of native rhizobacteria peculiar to selected food crops in Mabatho municipality of South Africa. Biological Agricult.&Horticult. 27:294-309.

3. Berge O., Heulin T., Achouak W., Richard C., Bally R., Balandreau J. 1991. *Rahnella aquatilis*, a nitrogen-fixing enteric bacterium associated with the rhizosphere of wheat and maize. Can. J. Microbiol. 37(3):195-203.

4. Bishop A., Davis R. 1990. Internal breakdown of onions caused by *Enterobacter cloacae*. Pl. Dis. 74:692-694.

5. Bradbury J. 1986. Guide to Plant Pathogenic Bacteria. CAB International. UK.

6. Escobar M., Carbonell G., Beriam L., Siqueira W., Yano T. 2001. Cytotoxin production in phytopathogenic and entomopathogenic *Serratia marcescens*. Rev. Latinoam. Microbiol. 43(4):165-170.

7. Hattingh M., Walters D. 1981. Stalk and leaf necrosis of onion caused by *Erwinia herbicola*. Pl. Dis. 65:615-618.

8. Holden N., Pritchard L., Toth I. 2009. Colonization outwith the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria. FEMS Microbiol. Rev. 33(4):689-703.

9. Innvista.com, n.d. [http://www.innvista.com/health/microbes/bacteria/entero.htm]

10. Kim K., Jordan D., McDonald G. 1998. *Enterobacter agglomerans*, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. Soil Biol.&Biochem. 30, 995–1003.

11. Klement Z., Rudolph K., Sands D. (Eds.) 1990. Methods in phytobacteriology. Budapest: Akademiai Kiado

12. Koch M., Taanami Z., Levy E. 1996. Damage to garlic crops caused by *Erwinia herbicola*. Phytoparasitica 24(2):125-126.

13. Lu J-J., Perng C-L., Lee S-Y., Wan C-C. 2000. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J. Clin. Microbiol. 38(6):2076–2080.

14. Müller H., Fanning G., Brenner D. 1995. Isolation of *Ewingella americana* from mollusks. Current Microbiol. 31(5):287-290.

15. OEPP/EPPO. 2006. *Erwinia chrysanthemi.* ListA2.

16. Schwartz H., Otto K. 2000. First report of a leaf blight and bulb decay of onion by *Pantoea ananitas* in Colorado. Plant Dis., 84:808.

17. Sellwood J., Lelliott R. 1978. Internal browning of hyacinth caused by *Erwinia rhapontici*. Pl. Pathol. 27:120-124.

18. Stoyanova M., Georgieva L., Badjakov I., Petrov N., Bogatzevska N. 2011. New pathogens of *Leucojum aestivum*. In: Scientific papers of the Jubilee Scientific Conference "The man and the universe", 728-734.

19. Stoyanova M., Moncheva P., Bogatzevska N. 2005. *Pseudomonas marginalis* and *P. fluorescens,* causal agents of soft rot of gladiolus, hippeastrum, onion, garlic and wild garlic bulbs. In: Scientific conference "Stara Zagora 2005", Vol. II, 220-226.

20. Van Baarlen P., Van Belkum A., Summerbell R., Crous P., Thomma B. 2007. Molecular mechanisms of pathogenicity: how do pathogenic microorganisms develop cross-kingdom host jumps? FEMS Microbiol. Rev. 31(3):239-277.