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IDENTIFICATION AND CHARACTERIZATION OF *PECTOBACTERIUM CAROTOVORUM*

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ABSTRACT

The present study aims to identify *Pectobacterium carotovorum*, known as one of the most common soft rot causative agents using classical and molecular methods. Four different *P. c.* subsp. *carotovorum* strains from various hosts were obtained, two of which are causative agents of soft rot disease in potato and the other two in onion or pepper. These strains were confirmed as *P. c.* subsp. *carotovorum* by microbial identification system (MIS). In addition, BIOLOG system was used to identify these strains and the results were found to be inconsistent with those of microbial identification system. Test results of hypersensitive reaction (HR), pathogenity and pectolytic activity proved that all strains were pathogens. A number of biochemical and molecular analyses (16S rDNA and specific PCR) were conducted for identification and characterization. The strains were identified in genus level by means of 16S rDNA PCR method. Identified as *P. c.* subsp. *carotovorum* according to MIS results, these four strains were analysed in specific PCR tests and found to give 434 bp-long band with Y1-Y2 primers. This is the first study to reveal that these four strains are causative agents of soft rot disease in potato, onion and pepper in East Anatolia and also in Turkey.

Key words: MIS and BIOLOG, PCR, *Pectobacterium carotovorum*, Soft rot.

INTRODUCTION

There are many diseases affecting cultured or wild plants and also innumerable causative agents of these diseases. Microorganisms play a major role in disease agents causing epidemics under suitable conditions, which result in considerable financial loss in the world Dolar *et al.* (2011).

Plant pathogens of the genus *Erwinia* form three main groups according to the symptoms they cause. The first group is *Amylovora* causing fire blight disease in fruit trees such as apple and pear. *Erwinia* amylovora is the most important species of *Amylovora* group. The second group is *Carotovora* consisting of causative agents of soft rot and black leg diseases. Members of this group are responsible for great loss in both field and storage rooms. *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium carotovorum* subsp. *atrosepticum* and *Dickeya chrysanthemi* are the most commonly known pathogens of Carotovora group. The third group, Herbicola consists of two main species: *Erwinia herbicola*, which is an epiphytic species and *Erwinia stewartii* that causes Stewart's wilt disease Leliott and Dickey (1984); Suparyono and Pataky (1989).

Identification can be described as establishing taxonomic status of classified microorganisms whereas characterization is to distinguish identified organisms on the basis of their characteristics. In general, identification methods are divided into two groups, i.e. classical and molecular methods. Classical methods are mainly based on phenotypic characteristics such as morphological, biochemical, physiological and pathological features. These methods have some disadvantages in terms of requiring alternative systems and excessive work force, and also being useful only at genus level and prone to give different results due to contamination or expertise Miller and Joaqium (1993). Although specific classical methods are employed for identification of some microorganisms, these methods are insufficient to identify and characterize the microorganisms. Therefore, classical methods should be supported by molecular methods. Currently, both classical and molecular methods are employed for identification and characterization of microorganisms.

Carbohydrates, lipids, proteins, DNA and RNA can be used for identification and characterization of microorganisms. Fatty acid analyses (Microbial Identification System (MIS)), metabolic enzyme profiling (BIOLOG), protein profiling (SDS-PAGE), serological reactions (Immunofloresans, Radioimmunoassay, Immuno Blot, Dot Immunobinding Assay and Enzyme Linked Immunosorbent Assay (ELISA)) and genetic profiling (rDNA-PCR, Rep-PCR, Eric-PCR, Box-PCR and Specific PCR) are used as molecular methods in identification and characterization of microorganisms Jackman (1985); Kersters (1985); Miller and Berger (1985); Miller and Martin (1988); Guillorit-Rondeau *et*

al.(1996); Scortichini *et al.* (1996); Zhang and Geider (1997).

MIS, BIOLOG, ELISA and PCR are the most commonly employed methods for identification of microorganisms and they are more preferable than classical methods because of reliability and velocity. However, both methods are insufficient in identification purposes on their own. Thus, molecular and classical methods should be used together for microbial identification.

The aim of this study is to make identification and characterization of bacterial strains, which are causative agents of soft rot disease for some plants (potato, onion and pepper), by using classical (morphological, cytological and biochemical tests) and molecular (MIS, BIOLOG, 16S rDNA PCR and specific PCR) methods.

MATERIALS AND METHODS

Isolation and Storage of Bacteria: Standard Trypticase Soy Broth Agar (TSBA), Nutrient Agar (NA) and Yeast Dextrose Carbonate Agar (YDC) media were used for isolation of bacteria. Infected plant materials were rinsed thoroughly under running and soaked 70% ethyl alcohol for 5 minutes for surface sterilization followed by rinsing with sdH_2O. Small pieces of plant material from both infected and healthy parts were cut off and soaked in sterilized tubes containing 2 ml sdH_2O for an hour and then 5-fold dilutions were prepared. Standard media were inoculated with 100 µl of each dilution and incubated at 25°C. Grown colonies were sub-cultured and purified. Purified strains were labeled and stored in 30% glycerol and Lauryl Broth (LB) (v/v, 1:1) at -86°C.

Identification of the bacterial strains by microbial identification system (MIS): Identification of the tested bacterial strains was confirmed by using microbial identification system (MIS). Preparation and analysis of FAMEs from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMEs were separated by gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm x 0,25 µm with crosslinked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package Miller (1982).

Identification of the bacterial strains by Biolog microplate system (BIOLOG): Identification of the tested bacterial strains was confirmed by using BIOLOG system. One or two days before the inoculation of Biolog GN2 and GP2 plates, bacterial strains were streaked on TSA or BUG agar plates. Each well of Biolog GN2 or GP2 micro-titer plates was inoculated with 125 µl of the Gram-negative or Gram-positive bacterial suspension with appropriate density (10^8 cfu/ml), respectively and incubated at 27 °C for 24 and 48 h. The development of color was automatically recorded using a microplate reader with a 590-nm wavelength filter. Identification (Biolog Microlog 34.20 database) and ASCII file output of test results, applying the automatic threshold option, were performed using BIOLOG420/ Databases/ GN601 and GP601 KID software Holmes *et al.* (1994). Carbon source utilization rates of the strains were estimated as percentages.

Hypersensitivity tests (HR): All of the bacterial strains were tested for hypersensitivity on tobacco plants (*Nicotina tabacum).* The bacterial suspension (10^8 cfu/ml) was prepared in sterile distilled water and infiltrated into the inter-costal area of the leaves of tobacco plants by using a 3-cc syringe (Becton Dickinson, Franklin Lakes, NJ, USA). The inoculated plants were incubated in a completely randomized design on the greenhouse bench for 24–48 h at 20–28 °C. The presence of rapid tissue necrosis at the inoculation site was recorded within 24–48 h after infiltration. This test was repeated, at least three times, for each strain. For HR tests, sterilized distilled water (sdH_2O) was used as a negative control Dadaşoğlu (2007).

Pathogenicity tests: Isolated bacteria were inoculated on TSA media for pathogenic test followed by 24 h incubation and bacterial suspension preparation at a concentration of 10^8 CFU/ml. The solutions were injected into fruits of host plants (potato, tomato, pepper, carrot, radish, melon, water melon, cucumber, zucchini, strawberry, apple, mulberry, and aubergine) placed in polyethylene bags with wet papers and these bags were followed for a week at 27-30°C.

For field trials, host plants for each pathogen from which it was isolated were grown. Bacterial suspensions at a concentration of 10^8 CFU/ml were inoculated into plant parts above the ground and the plants were covered by polyethylene bags to maintain humidity condition for 2 days. After then, the bags were discarded and the plants were monitored whether developing soft rot disease during 15 days. As control solution, sdH_2O was used.

Pectolytic activity tests: Fresh and healthy potato tubers were sterilized in 5% sodium hypochlorite for 10 minutes. Sterilized tubers were sliced 5 mm diameter and placed onto petri dishes containing sterilized wet papers. Bacterial broth cultures of 24-hour incubation were injected into potato slices in 1 ml volume and these slices were incubated at 26±2°C. After 24-72 h observed softness was assessed as positive result. As a control solution, sdH_2O was used.

Phenotypic tests: Strains characterized as positive in pectolytic activity were submitted for the conventional biochemical and physiological tests including Gram reaction, fermentative metabolism Hugh and Leifson (1953) and oxidase and catalase activity. Production of reducing substances from sucrose, indole production from tryptophan, were studied using previously described methods Gallois *et al.* (1992); Gardan *et al.* (2003). They were also checked for production of phosphatase and amilase, sensitivity to erythromycin Schaad *et al.* (2001), ability to grow at 37°C in nutrient broth and growth in 5% sodium chloride on nutrient agar at 28°C. In addition, the assimilation of carbon sources were tested on the basal medium of Ayers *et al.* (1919) supplemented with 1% carbohydrates including lactose, glucose, fructose and mannitol.

Bacterial DNA extraction: Genomic DNA of bacterial strains was extracted according to the methods described by Lazo *et al.* (1987); Ausubel *et al.* (1994); Khoodoo and Jaufeerally-Fakim (2004) with some modifications.

PCR amplification of 16S rDNA: PCR conditions, forward and reverse primers for 16S rDNA amplification were shown in Table 2.1.

Genetic profiling by specific-PCR: Specific-PCR method was employed for the strains of *P. c.* ssp. *carotovorum.* PCR conditions and preparation of master mix were shown in Table 2.1.

Electrophoresis of PCR amplicons: PCR amplicons for 16S rDNA and specific-PCR were checked in 1% agarose gel prepared with 0.5XTBE buffer while amplicons for REP, ERIC and BOX-PCR reactions were checked in 1.5% agarose gel. Electrophoresis was conducted in 70V for 2.5-3 h. Gels were analyzed in an image analysis system.

Table 2.1. PCR conditions used for identification of bacterial strains

PCR method	Primers	Master mix (per sample)		PCR programme	
specific PCR: ***Pectobacterium carotovorum subsp. carotovorum***	**Y1:** TTA CCG GAC GCC GAG CTG TGG CGT Y2: **5CAG GAA GAT GTC GTT ATC GCG AGT**	10 x PCR buffer	5 µl		94°C for 5 min
		25 mM $MgCl_2$	3 µl	**1.** Denaturation a	94°C for 30 s
		dNTP	0,5 µl	**2.** Denaturation b	55°C for 45 s
		Primers	0.3 µl	**3.** Annealing	72°C for 45 s
		Taq DNA polymerase (5U)	0.25 µl	**4.** Extension	34 repeat
		sdH_2O	37,65 µl	**5.** Cycle (2, 3, 4)	**72°C for 7 min**
		Template DNA (50 ng/µl)	3 µl	6. **Extension**	
16S rDNA PCR: (*Pectobacter* sp.,)	**fD1:** AGAGTTTGATCCTGG CTCAG **rP2:**ACGGCTACCTmö kkk-TGTTACGACTT	10 x PCR buffer	5 µl	**1.** Denaturation a	94°C for 4 min
		dNTP	1 µl	**2.** Denaturation b	94°C for 1 min
		Primers	0.3 µl	**3.** Annealing	58°C for 1 min
		Taq DNA polymerase (5U)	0.5 µl	**4.** Extension	72°C for 3 min
		sdH_2O	39.9 µl	**5.** Cycle (2, 3, 4)	35 repeat
		Template DNA (50 ng/µl)	3 µl	**6.** Extension	72°C for 10 min

RESULTS

The results obtained from MIS, BIOLOG, hypersensitivity, pathogeny and pectolytic activity tests for the strains identified as causative agents of soft rot disease are shown in Table 3.1. All the strains analyzed in microbial identification system (MIS) were tested for hypersensitivity in tobacco plant (*Nicotina tabacum* L. var. Samsun) and all gave positive results. In addition, these strains were also tested positive for pathogeny and pectolytic activity tests.

According to microbial identification system, all strains were determined to be *P. carotovorum* species whereas the results of BIOLOG system showed that only the strain F-391 belongs to *P. carotovorum* while F-11 is identified as Salmonella typhimurium, F-37 is *Enterobacter cloacae* and F-680 is *Escherichia coli*.

The results obtained from morphological and biochemical tests are shown in the Table 3.2. The strains tested positive for hypersensitivity, pectolytic activity and pathogeny test for the host which the strain was isolated from have rod-like shape morphologically. Pathogen strains were found to be motile in examination on concave microscope slide. All the pathogen strains grew on nutrient agar at 37°C. The strains were tested for positive for catalase test while negative for oxidase and potassium oxidase. The strain F-37 was positive for erythromycin sensitivity test while the other strains were tested negative. All the strains have the ability to grow on media with 5% NaCl. The strains were proved to be positive for phosphatase while they were found negative for amylase and indole production. In addition, the strains were not able to reduce sucrose. All the strains were tested positive for acide production from glucose, fructose, lactose and mannitol with the exception of F-391 which was negative for acid production from glucose.

PCR Results: 16S rDNA PCR Amplification: All the strains yielded 1400-1500 bp long fragments with 16S rDNA amplification.

Specific PCR Amplification: The results obtained from specific PCR tests are shown in the Figure 1.The strains F-18, F-37, F-391 and F-680 identified as *P. carotovorum* according to microbial identification system yielded 434 bp long fragment with Y1 and Y2 primers specific to *P. carotovorum*.

Table 3.1.Characterization of pathogen bacteria identified by MIS and BIOLOG and data on isolation source and test results for HR, pathogeny and pectolytic activity.

SN	MIS identification result	MBI	BIOLOG identification result	BBI	K	BM	L	HR	P	PA
F-18	*Pectobacterium caratovorum* subsp. *carotovorum*	0.79	*Salmonella typhimurium*	0.47	Pepper	Fruit	Artvin	+	+	+
F-37	*P. c.*subsp. *carotovorum*	0.76	*Enterobacter cloacae*	0.40	Potato	Tuber	Şenkaya	+	+	+
F-391	*P. c.* subsp. *carotovorum*	0.79	*Pectobacterium carotovorum* subsp. *atrosepticum*	0.44	Onion	Stem	Hınıs	+	+	+
F-680	*P. c.* subsp. *carotovorum*	0.64	*Escherichia coli*	0.15	Potato	Stem	Pasinler	+	+	+

SN: Strain no**, MBI:** MIS similarity index, **BBI:** BIOLOG similarity index, **K:** Host, **BM:** Plant material, **L:** Locality, **HR:** Hypersensitivity test, **P:**Pathogenity, **PA:** Pectolytic activity, **+ :** Positive, **-:** Negative

Table 3.2. Morphological and biochemical test results.

Strain no	MIS identification	BIOLOG identification	Motility	Cell shape	Colony colour	Growth at 37	Catalase	Oxidase	KOH	Sensitivity to ervthromvcin	Tolerance to 5% NaCl	Phosphatase activity	Amylase activity	Indole production	Sucrose reduction	Acid production from carbohydrates			
																Glucose	Fructose	Lactose	Mannitol
F-18	*P. c.* subsp.*carotovorum*	*S. typhimurium*	+	R	LC	+	+	-	-	-	+	+	-	-	-	+	+	+	+
F-37	*P.c.* subsp. *carotovorum*	*E. cloacae*	+	R	LC	+	+	-	-	+	+	+	-	-	-	+	+	+	+
F-391	*P. c.* subsp.*carotovorum*	*P. c.* subsp. *atrosepticum*	+	R	LC	+	+	-	-	-	+	+	-	-	-	-	+	+	+
F-680	*P. c.* subsp. *carotovorum*	*E. coli*	+	R	LY	+	+	-	-	-	+	+	-	-	-	+	+	+	+

LC: Light cream, **LY:** Light yellow, **R:** Rod, **+:** Positive, **-:**Negative

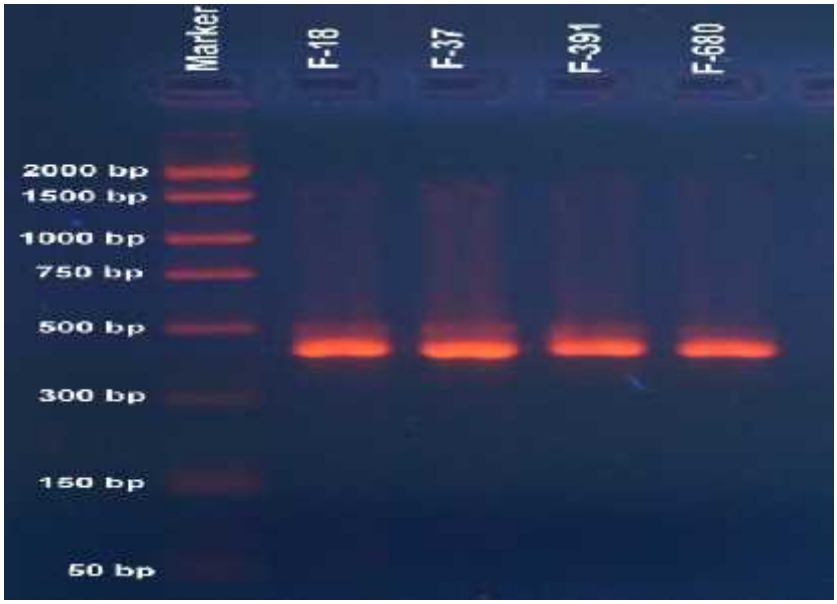

Figure 1. Specific PCR test results

DISCUSSION

Bacterial agents for soft rot disease cause watery lesions, rot, tissue softening and wilt on various parts of host plants such as tuber, stem, fruit, branch and leaves Aysan *et al.* (2003); Fiori and Schiaffino (2004); Boyraz *et al.* (2006); Umunna and Austin (2016); Ozturk and Aksoy (2017); Wang *et al.* (2017). These pathogens lead to great financial loss of approximately 50-100 million dollars annually due to severe infections especially in culture plants. Among the plant pathogens, the genera *Pectobacterium, Pseudomonas, Enterobacter, Bacillus, Clostridium and Flavobacterium* have some species responsible for both soft rot and other diseases worldwide Prombelon and Kelman (1980). In this study, *Pectobacterium* spp. were observed to be causing soft rot disease on various host tissues such as stem, tuber and fruit.

Most of the studies on soft rot disease in our country focused more on Mediterranean region by detecting *E. c.* subsp. *carotovora*, *E. chrysanthemi* and *P. viridiflava* as causative agents of the disease Aysan *et al.* (2005); Saygılı *et al.* (2005); Mirik *et al.* (2011). Similarly, there are also other studies from different regions related to pathogeny of *P. carotovorum* and *P. atrosepticum* for soft rot disease Boyraz *et al.* (2006); Bastas *et al.* (2009); Ustun and Arslan (2016); Fujimoto *et al.* (2017). However, this study is the first to seek causative agents for soft rot disease in this region.

By using microbial identification system, the strains were identified initially so the other analyses were planned more easily and reliably. The strains identified by MIS were tested positive for HR. Some researchers are reported to state that these strains gave positive or negative results for hypersensitivity tests on tobacco plants Gallelli *et al.* (2009); Pitman *et al.* (2010); Mikicinski *et al.* (2010). Thus, all these strains identified as *P. carotovorum* subsp. *carotovorum* by MIS analysis and the strains with positive HR results in the literature were tested for pathogeny on their host plants. These pathogens were mostly collected from fruit and tuber tissues which is consistent with the previous research Saygılı *et al.* (2005); Maisuria and Nerurkar (2012); Van Vaerenbergh *et al.* (2012); Oskiera *et al.* (2017).

Most bacterial strains are capable of producing enzymes causing diseases in plant tissues while a few are causative agents for soft rot disease Prombelon and Kelman (1980). Pectolytic enzymes produced by bacterial strains are of great significance because these enzymes hydrolyse pectin in cell wall structure of host tissues. In this study, all pathogen strains were analysed for pectolytic activity. In previous works, these strains were reported to be positive for pectolytic activity on potato slices Ngadze *et al.*(2012); Crepin *et al.*(2012).

Morphological and biochemical characteristics of the strains such as shape and motility were consistent with those in the literature Brenner *et al.* (2007). All the strains are Gram-negative and grow at 37°C. *Pectobacterium* strains are capable of growing at 25-30°C optimally and 40°C maximally, which is consistent with our results Brenner *et al.* (2007). All strains were tested positive for catalase test while negative for oxidase test.

The strain F-37 was found to be sensitive to erythromycin. All strains have tolerance for 5% NaCl and grow normally. However, some species were reported to be sensitive to 5% NaCl Ngadze *et al.* (2012). For phosphatase test, reports show both positive and negative results of these species Palacio-Bielsa *et. al.* (2010); Tavasoli *et al.* (2011); Sarris *et al.* (2011). In our study, all the strains were tested positive for phosphatase and this result is consistent with the literature. All the strains are capable of reducing sucrose except the strain *P. c.* subsp. *carotovorum* (F-391). Various results for sucrose reduction were reported in the literature Gallelli *et al.* (2009); Van Der Merwe (2009); Ashmawy *et al.* (2015). In previous works, a number of carbohydrates such as sucrose, mannitol, glucose, fructose, lactose, mellibiose and sorbitol were applied to determine acid production of these species Dickey (1978); Sarris *et al.* (2011); Rahman *et al.* (2012); Maisuria and Nerurkar (2012). Four carbohydrates, i.e. glucose, fructose, lactose and mannitol, were used for acid production test. Because members of the genus *Pectobacter* were known to be the most important causative agents for soft rot disease worldwide, a variety of morphological and biochemical tests having the utmost importance for identification of these pathogens were performed in this work. Our results for morphological and biochemical tests are consistent with those in the literature Aysan *et al.* (2005); Boyraz *et al.* (2006); Van Der Merwe (2009); Maisuria and Nerurkar (2012); Ashmawy *et al.* (2015).

Polymerase chain reaction, which is one of the most commonly used molecular methods for identification of microorganisms, is a special and reliable method using template nucleic acid molecules from pathogenic agents such as bacteria, viruses, fungi, parasites and protozoa with primers and heat-resistant enzymes Schochetman and Jones (1988). It was revealed in 1980s that phylogenetic relationships of bacteria and other life forms can be shown by comparing conserved DNA regions Woese *et al.* (1985). Being one of the most conserved molecular clocks, 16S rDNA is applied to show phylogenetic relationships among bacteria Kimura (1980); Pace (1997); Harmsen and Karch (2004).

In this work, universal primers of fD1 and rP2 were used for PCR amplification of 16S rRNA gene region and nearly 1500 bp long amplicons were obtained as in previous reports of 16S rDNA-PCR for *Pectobacter* Kang *et al.* (2003); Rattanasuk and Ketudat-Cairns (2009); Santana *et al.* (2012).

Four strains of *P. c. subsp. carotovorum* yielded 434 bp long amplicons with the primers Y1 and Y2 for specific PCR tests which is consistent with the literature Yahiaoui-Zaidi *et al.* (2003); Phokum *et al.* (2006).

Bacterial causative agents of soft rot disease for potato, onion and pepper were identified and characterized by classical (morphological, cytological and biochemical tests) and molecular (MIS, BIOLOG, 16S rDNA PCR and specific PCR) methods. Our results confirmed that all strains are members of *P. c.* subsp. *carotovorum*. This is the first study revealing these strains were isolated from these culture plant hosts in Turkey and will contribute to knowledge of causative agents of soft rot disease in agriculture.

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REFERENCES

Ashmawy, N.A., N.M. Jadalla, A.A. Shoeib and A. F. El-Bebany (2015). Identification and Genetic Characterization of *Pectobacterium* spp. and Related *Enterobacteriaceae* Causing Potato Soft Rot Diseases in Egypt. J. Pure Applied Microbiology. 9(3): 1847-1858.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (1994). Current protocols in molecular biology. John Wiley and Sons, New York. 2.0.1–2.14.8

Ayers S.H., P. Rupp and W.T. Johnson (1919). A study of the alkali forming bacteria. Milk USDA Bull. 782.

Aysan, Y., A. Karatas and O. Cinar (2003). Biological control of bacterial stem rot caused by *Erwinia chrysanthemi* on tomato. Crop Protection 22(6): 807-811.

Aysan, Y., F. Sahin, R. Cetinkaya-Yildiz, M. Mirik and Y. Yucel (2005). Occurence and primer inoculum sources of bacterial stem rot caused by *Erwinia* species on tomato in the eastern Mediterraanean region of Turkey. J. Plant Diseases and Protection. 112(1): 42-51.

Bastas, K. K., H. Hekimhan, S. Maden and M. Tör (2009). First report of bacterial stalk and head rot disease caused by *Pectobacterium atrosepticum* on sunflower in Turkey. Plant Disease 93(12): 1352.

Boyraz, N., K.K. Bastas,S. Maden and A. Yasar (2006). Bacterial leaf and peduncle soft rot caused by *Pectobacterium carotovorum* on Tulips in Konya, Turkey. Phytoparasitica, 34(3): 272-280.

Brenner, J.D., N.R. Kieg and M.G. Garrity (2007). *Bergey's* Manual of Systematic Bacteriology. 2nded. Williams & Wilkins Co. Baltimore (USA). p, 721-733.

Crepin, A., C. Barbey, A. Cirou, M. Tannieres, N. Orange,M. Feuilloley, Y. Dessaux, J.F. Burini, D. Faure and X. Latour (2012). Biological control of pathogen communication in the rhizosphere: A novel approach applied to potato soft rot due to *Pectobacterium atrosepticum*. Plant Soil. 358: 27-37.

Dadaşoğlu, F. (2007). Isolation and identification of bacterial strains with insecticidal activities against greenhouse and field pest. Graduate School of Natural and Applied Science Institute, Department of Plant Protection, Master Thesis,Atatürk University, Turkey.

Dickey, R.S. and A. Kelman (1988). *Erwinia*: "*Carotovora*" or soft rot group. In: Laboratory Guide for Identification of plant pathogenic bacteria.p. Schaad, N.W. 2 ed. St. Paul: APS. p.44-59.

Dolar, F.S., E. Demirci, H. Basım, F. Demirci and İ.E. Elibüyük (2011). Phytopathology. Anadolu University (Eskişehir)199.

Fiori, M. and A. Schiaffino (2004). Bacterial Stem Rot in Greenhouse Pepper (*Capsicum annuum* L.) in Sardinia (Italy): Occurrence of *Erwinia carotovora* subsp. *carotovora.* J. Phtopathology, 152(1): 28-33.

Fujimoto, T., S. Yasuoka, Y. Aono, T. Nakayama, T. Ohki, M. Sayama and T. Maoka (2017). First Report of Potato Blackleg Caused by *Pectobacterium carotovorum* subsp *brasiliense* in Japan. Plant Disease. 101(1): 241-242.

Gallelli, A., M. Galli, D. De Simone, M. Zaccardelli and S. Loreti (2009). Phenotypic and Genetic Varıability of *Pectobacterium carotovorum* Isolated from Artichoke in the Sele Valley. J. Plant Pathology, 91(3): 757-761.

Gallois, A., R. Samson, E. Ageron and P.A.D. Grimont (1992). *Erwinia carotovora* subsp. *odorifera* subsp. nov., associated with odorous soft rot of chicory (*Cichorium intybus* L.). Int J Syst Bacteriol 42: 582–588.

Gardan, L., C. Gouy,R. Christen andR. Samson (2003). Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. International J. Systematic and Evolutionary Microbiology 53: 381-391.

Guillorit-Rondeau, C., L. Malandrin and R. Samson (1996). Identification of two serological flagellar types (H1 and H2) in *Pseudomonas syringae*

pathovars. European J. Plant Pathology, 102: 99-104.

Harmsen, D. and H. Karch (2004). 16S rDNA for diagnosing pathogens: a living tree. ASM News. 70:19–24.

Holmes, B., M. Costas, M. Ganner, S.L.W. On and M. Stevens (1994). Evaluation of biolog system for identification of some gram-negative bacteria of clinical importance. J. Clinical Microbiology. 1970-1975.

Hugh, R. and E. Leifson (1953). The taxonomic significance of fermentative vs. Oxidative metabolism of carbohydrates by various Gram negative bacteria. J. Bact. 66: 24.

Jackman, P.J.H. (1985). Bacterial taxonomy based on electrophoretic whole-cell protein patterns. The Society for Applied Bacteriology. 415-429.

Kang, H.W., S.W. Kwon and S.J. Go (2003). PCR-based specific and sensitive detection of *Pectobacterium carotovorum* ssp. *carotovorum* by primers generated from a URP-PCR fingerprinting-derived polymorphic band. Plant pathol 52: 127-133.

Kersters, K., (1985). Numerical methods in the classification of bacteria protein electrophoresis. Society for General Microbiology. 337-368.

Khoodoo, M.H.R. and Y. Juafeerally-Fakim (2004). RAPD-PCR fingerprinting and Southern analysis of *Xanthomonas axonopodis* pv. *dieffenbachiae* strains isolated from different aroid hosts and locations. Plant Dis. 88:980–988.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences". J. Molecular Evolution. 16(2): 111–120.

Lazo, G.R. and D.W. Gabriel (1987). Conservation of plasmid DNA sequences and pathovar identification of strains of *Xanthomonas campestris*. Phytopathology. 77:448-453.

Lelliott, R.A. and R.S. Dickey (1984). Genus VII. *Erwinia*. In: Bergey's manual of systematic bacteriology, p. 469-476. Williams and Wilkins, Baltimore(USA).

Maisuria, V.B. and A.S. Nerurkar (2012). Characterisation and differantiation of soft rot causing *Pectobacterium carotovorum* of Indian origin. Eur. J. Plant Pathol. 136(1): 87-102.

Mikicinski, A., P. Sobiczewski, M. Sulikowska, J. Pulawska and J. Treder (2010). Pectolytic bacteria associated with soft rot of calla lily (*Zantedeschia* spp.) tubers. J. Phytopathology 158: 201-209.

Miller, L.T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J. Clin. Microbiol, 16: 584-586.

Miller, I. and T. Berger (1985). Bacteria identification by gas chromatography of whole cell fatty acids. Hewlett-Packard Gas Chromatography Application Note, Hewlett-Packard Co., Alto, CA., 228-238.

Miller, S.A. and T.R. Joaquim (1993). Diagnostic techniques for plant pathogens. Biotechnology in Plant Disease Control, 17: 321-339.

Miller, S.A. and R.R. Martin (1988). Molecular Diagnosis of plant disease. Phytopathology, 26: 409-432.

Mirik, M., Y. Aysan and F. Sahin (2011). Characterization of *Pseudomonas cichorii* isolated from different hosts in Turkey. Int. J. Agriculture and Biology. 13(2): 203-209.

Ngadze, E., C.L. Brady, T.A. Coutinho and J.E. van der Waals (2012). Pectinolytic bacteria associated with potato soft rot and blackleg in South Africa and Zimbabwe. European J. Plant Pathology, 134:533–549.

Oskiera, M., M. Kaluzna, B. Kowalska and U. Smolinska (2017). *Pectobacterium carotovorum* subsp. *Odorıierum* on Cabbage and Chinese Cabbage: Identification, Characterization And Taxonomic Relatedness of Bacterial Soft Rot Causal Agents. J. Plant Pathology. 99(1): 172-185.

Ozturk, M. And H.M. Aksoy (2016). First Report of *Pectobacterium carotovorum* subsp. *brasiliense* Causıng Blackleg and Soft Rot of Potato in Turkey. J. Plant Pathology. 98(3): 677-697.

Pace, N. (1995). A Molecular View of Microbial Diversity and the Biosphere. Science, 276(5313): 734-740.

Palacio-Bielsa, J., M.A. Cambra and M.M. Lopez (2006). Characterisation of potato isolates of *Dickeya chrysanthemi* in Spain by a microtitre system for biovar determination. Annals of Applied Biology. 148: 157-164.

Perombelon, M.C.M. and A. Kelman (1980). Ecology of the Soft Rot Erwinias. Ann. Rev. Phytopathol. 18: 361-387.

Phokum, C., P. Jitareeat, S. Phochananchai and S. Cheevadhanarak (2006). Detection and classification of soft rot erwinia of vegetables in Thailand by DNA polymerase chain reaction. Acta horticulturae. 712: 917-926.

Pitman, A.R., S.A. Harrow and S.B. Visnovsky (2010). Genetic characterisation of Pectobacterium wasabiae causing soft rot disease of potato in New Zealand. Eur J Plant Pathol. 126:423–435.

Rahman, M.M., M. Eaqub-Ali, A.A. Khan, U. Hashim, A.M. Akanda and M.A. Hakim (2012). Characterization and identification of soft rot bacterial pathogens in Bangladeshi potatoes. African J. Micro. Res. 6(7): 1437-1445.

Rattanasuk, S. and M. Ketudat-Cairns (2009). *Chryseobacterium indologenes*, novel mannanase producing bacteria. J. of Sci and Tech 31(4): 395-399.

Santana, M.A., M. Rodrıguez, J. Matehus, J. Faks, A. Bocsanczy, A. Gerstl, G. Romay, J. Montilla, C.E.Fernande, N. Moreno Zambrano and D. Marval (2012). A New Bacterial Disease of Cassava in Venezuela Caused by *Enterobacter cloacae*. International J. Agri. Biology. 14(2): 183-189.

Sarris, P. F., E. Trantas, M. Pagoulatou, D. Stavrou, F. Ververidis and D.E. Goumas (2011). First report of potato blackleg caused by biovar 3 *Dickeya* sp. (*Pectobacterium chrysanthemi*) in Greece. New Disease Reports, 24: 21.

Saygılı, H., Y. Aysan, F. Sahin, M. Mirik and N. Ustun (2005). Phenotypic characterization of tomato pith necrosis pathogens in Turkey. APS Annual Meeting, July 30-Agust 3, 2005, Austin, Texas(USA).

Schaad, N.W., J.B. Jonesand and W. Chun, (2001). Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3th. Revista Mexicana de Fıtopatologıa/ 343 ed. APS Press. St. Paul, Minnesota (USA), p. 373.

Schochetman G., C.Y. Ou and W.K. Jones (1988). Polymerase chain reaction. J Infect Dis. 158:1154-7.

Scortichini, M., J.D. Janse, M.P. Rossi and J.H.J. Derks (1996). Characterization of *Xanthomonas campestris* pv. *pruni* strains from different host by pathogenicity test and analysis of whole-cell fatty acids and whole-cell proteins. J. Phytopathology, 144: 69-74.

Suparyono Pataky, J.K. (1989). Influence of host resistance and growth stage at the time of inoculation on Stewart's wilt and Goss' wilt development and sweetcorn hybrid yield. Plant Disease. 73: 339-345.

Tavasoli, E., A.R. Marafat and N. Hassazadah (2011). Identity and genetic diversity of *Pectobacterium* spp., causal agents of potato soft rot in Zanjan,Iran. African J. Plant Science. 5(6): 329-336.

Umunna, O.E. and A.A. Austin (2016).An Overview of Characterization and Identification of Soft Rot Bacterium *Erwinia* in Some Vegetable Crops. Greener J. Biological Sciences. 6(3): 46-55.

Ustun, N. and N. Arslan (2016). Bacterial Stem Rot of Globe Artichoke (*Cynara cardunculus* var. *scolymus*) Caused by Pectobacterium carotovorum subsp carotovorum in Turkey. J. Plant Pathology. 98(1): 91-96.

Van der Merwe, J.J., T. A. Coutinho, L. Korsten and J.E. van der Waals (2010). *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg on potatoes in South Africa. Eur J Plant Pathol 126:175–185.

Van Vaerenbergh, J., S. Baeyen, P. De Vos and M. Maes (2012). Sequence Diversity in the *Dickeya flic* Gene: Phylogeny of the *Dickeya* Genus and TaqMan® PCR for *'D. solani'*, New Biovar 3 Variant on Potato in Europe. PLoS ONE 7(5): 357-380.

Yahiaoui-Zaidi, R., B. Jouan and D. Andrivon (2003). Biochemical and molecular diversity among *Erwinia* isolates from potato in Algeria. 52(1): 28-40.

Wang, J., Y. Wang, T. Zhao, P. Dai and X. Li (2017). Characterization of the pathogen causing a new bacterial vein rot disease in tobacco in China. Crop Protection. 92: 93-98.

Zhang, Y. and K. Geider (1997). Differentiation of *Erwinia amylovora* strains by pulsed-field gel electrophoresis. Applied and Enviromental Microbiology. 63: 4421-4426.