Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 27 (2):234-240, 2022
(*Mustafa Kemal University Journal of Agricultural Sciences* 27 (2):234-240, 2022)
e-ISSN: 2667-7733
http://dergipark.org.tr/mkutbd

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of the host range of *Pectobacterium polaris* causing bacterial soft rot disease

Bakteriyel yumuşak çürüklük hastalığına neden olan *Pectobacterium polaris*'in konukçu aralığının belirlenmesi

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MAKALE BİLGİSİ / ARTICLE INFO

Makale tarihçesi / Article history:
DOI: 10.37908/mkutbd.1064147
Geliş tarihi /Received:05.02.2022
Kabul tarihi/Accepted:09.04.2022

Keywords:
Bacterial disease, *Pectobacterium polaris*, host range, pathogenicity.

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Ö Z E T / A B S T R A C T

Aims: *Pectobacterium polaris* is a novel species of *Pectobacterium* taxa, which was reported in different regions of the world and has been recently detected in Turkey. In this study, in order to determine the host range of this novel species, two representative strains were evaluated in terms of their pathogenicity on several host plants, and it is aimed to determine plant species under the risk of the pathogen.
Methods and Results: *P. polaris* strains NV3 and NV19, isolated from potato plants showing blackleg symptoms in Nevşehir province during the vegetation period of 2019, were used for artificial inoculation. Bacterial suspension of both strains was inoculated by injecting into the red cabbage, broccoli, cauliflower, onion, garlic, carrot, tomato, pepper, eggplant, cucumber, squash, melon, watermelon, lettuce, sunflower and sugar beet plants. The appearance of the disease symptoms indicated that both strains of *P. polaris* caused disease in all the plant species inoculated artificially. Viable bacterial colonies were re-isolated from infected plant tissue.
Conclusions: *Pectobacterium* species are very destructive bacterial plant pathogens that cause soft rot on numerous plant species. It has been determined that *P. polaris*, which has been reported in different regions of the world and in Turkey as a new emerging pathogen within in a wide host range. Disease protection precautions should be taken in the regions where the pathogen is isolated for the first time, and especially pathogen transmission should be prevented to the clean production areas by potato tubers.
Significance and Impact of the Study: All tested plant species were determined as potential hosts for *P. polaris*. The risk status of the pathogen on these plants, which have economic importance in Turkey, was determined for the first time with this study

Atıf / Citation: Öztürk M (2022) Determination of the host range of *Pectobacterium polaris* causing bacterial soft rot disease. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi,* 27(2) : 234-240. DOI: 10.37908/mkutbd.1064147

INTRODUCTION

The Pectobacteriaceae family includes several significant bacterial plant pathogens that are able to cause diseases in different plant species worldwide. It comprises the *Pectobacterium* and *Dickeya* spp. (formerly classified as pectinolytic *Erwinia* spp.) which cause soft rot diseases in a wide range of plants including potato (Gardan et al., 2003; Samson et al., 2005). Both genera are gram-negative (-), pectinolytic facultative anaerobic, rod-shaped and peritric flagellated bacteria with 1-3 µm cells (Perombelon and Kelman, 1980; Charkowski, 2007, Czajkowski et al., 2015).

They are capable of secreting and synthesizing the plant cell wall degrading enzymes (Hélias et al., 2012) and are separated by forming cavities in the Crystal Violet Pectate (CVP) medium supplemented with pectate source. Annual losses in certification systems due to the infections caused by *Pectobacterium* and *Dickeya* spp. is reported as 30 million Euros in the Netherlands, which is an important seed potato exporter (Czajkowski et al., 2015).

Pectobacterium species are highly heterogeneous and cause soft rot disease during field, harvest, storage or transportation process (Pérombelon, 2002). On tuber plants such as potato, carrot, beet, etc., the most predominant and widely reported pathogenic species of the genus are *P. atrosepticum*, *P. brasiliense*, *P. carotovorum* and *P. parmentieri* around the world. (Gardan et al., 2003; Duarte et al., 2004; De Boer et al., 2012; Waleron et al., 2013; Khayi et al., 2016; Ozturk et al., 2018; van der Wolf et al., 2021; Öztürk and Soylu, 2022; Soylu et al., 2022).

Taxonomy of *Pectobacterium* was systematically evaluated by 16S rDNA, DNA-DNA hybridization, and sequencing housekeeping genes (Waleron et al., 2002; Duarte et al., 2004; Nabhan et al., 2012). Superior discrimination ability of advanced diagnostic techniques, especially whole-genome analysis, has led to the classification of strains with different biochemical and physiological characteristics into novel species (Oulghazi et al., 2020; Portier et al., 2019). Strains obtained in the past have been re-identified according to current techniques, and some atypical strains have been assigned to newly established species of *Pectobacterium* spp. (Lee et al., 2014; Waleron et al., 2014). According to the present taxonomy, the *Pectobacterium* genus now includes 18 species (Oulghazi et al., 2020; Portier et al. 2019; Waleron et al., 2019a).

As one of these species, *P. polaris* strains were suggested to represent a novel species of the genus by re-identification of two soft-rot-causing strains, which were isolated from potato tubers showing severe symptoms from a latently infected tuber in Norway (Dees et al., 2017). Waleron et al. (2019b) also re-identified 250 strains isolated from plants with soft rot symptoms since 1995 in Poland and classified 5 of the strains from potato (n=4) and from bittersweet (n=1) as *P. polaris*, which were previously identified as *P. carotovorum* subsp. *carotovorum*. Chen et al. (2021) isolated four pectinolytic bacterial strains from soft-rotted Chinese cabbage in China and identified them as *P. polaris*. Sarfraz et al. (2019) identified two strains as *P. polaris* caused potato blackleg disease in Pakistan. Voronina et al. 2019 assigned five strains to *P. polaris* as a new pathogenic species of potato in Russia and provided the evidence that a previously unreported pathogen was present in the surveyed fields from a period of 2012 to 2019. In Morocco, 5% of pectinolytic strains (n=119) were identified as *P. polaris* from diseased potato samples (Oulghazi et al., 2020). Jee et al. (2020) reported that the pathogen constituted a 0.9% portion of 225 isolated *Pectobacterium* strains in South Korea. Wang et al. (2021) revealed that the bacterium caused aerial stem rot of potato where the disease is a significant threat to the local potato industry in China.

This study aimed to determine the host range of *P. polaris* strains. These results confirmed for the first time the pathogenicity and ability of Turkish *P. polaris* strains to cause disease on several agriculturally and economically important commercial plants in Turkey.

MATERIALS and METHODS

Bacterial strains used in the study

The strains (NV3 and NV19) were previously obtained from stem tissue of blackleg showing potato plants collected in Nevşehir province during the vegetation period of 2019 (Öztürk, 2022). Both strains were identified as *P. polaris* by biochemical, physiological, pathogenicity and housekeeping gene sequencing of *mdh* and *rec*A genes (Waleron et al., 2002; Ma et al., 2007). The cultures were stored at -20°C for long-term usage in the phytobacterial collection of the Department of Plant Protection, Yozgat Bozok University, Yozgat, Turkey. Cultures were grown routinely on Nutrient agar medium (NA) and incubated for 24-48 hours at 28°C for the experiments.

Pectinolytic activity test

Before host range assay, maceration ability of the strains was confirmed by inoculating them on potato tuber slices in pectinolytic activity test. For this purpose, fresh and disease-free potato tuber (cv. Marabel) was sterilized by soaking in 5% sodium hypochlorite for 10 minutes. The sterilized tuber was sliced approximately 5 mm in thickness and placed in a petri dish containing sterile moistened paper. Stock cultures of strains were streaked onto NA medium. Pure colonies were harvested from agar plates by sterile loop and inoculated onto the potato slices, and then they were incubated at 28°C for 48 h. Sterilized water (dH_2O) was used for negative control (Lojkowska et al., 1995).

Preparation of bacterial inoculum

For the preparation of bacterial suspension, a single colony was suspended in sterile distilled water, and adjusted to McFarland 0.5 value, which is equivalent to $2x10^7$ cfu ml^{-1} (Oztürk et al., 2021).

Host range assays

Commercial seed varieties of red cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* DC), broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleraceae* var. *botrytis*), onion (*Allium cepa*), garlic (*Allium sativum*), carrot (*Daucus carota*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), melon (*Cucumis melo*), watermelon (*Citrillus lanatus*), lettuce (*Lactuca sativa*), sunflower (*Helianthus annuus*) and sugar beet (*Beta vulgaris*) were tested for host range of the studied pathogen. Seed materials were surface disinfected in 1% sodium hypochlorite (NaOCl) for 1-2 minutes, and rinsed in sterile water. Seeds were sown in plastic pots filled with sterile peat. Plants were grown for 14-21 days in 16 hours of light at 25°C and 8 hours of darkness at 16°C. Bacterial suspension of strains (20 µl) was infiltrated into stem tissue of red cabbage, broccoli, cauliflower, tomato, pepper, eggplant, cucumber, squash, melon, watermelon and sunflower, and leaves of onion, garlic, carrot, lettuce and sugar beet plants using a hypodermic syringe. The experiment was conducted for three replicates per strain for each plant species and repeated two times.

Re-isolation of bacterial strains from infected plant tissues

Artificially inoculated plants showing disease symptoms triggered by strains were subjected to bacterial re-isolation. Small pieces of infected tissue (3-5) were aseptically removed, surface-sterilized (treated with 70% ethanol for 40 s) and placed into a tube with 0.3 ml of sterile saline solution, and left to soak for 30 minutes. The suspensions were streaked onto crystal violet pectate (CVP) medium and incubated at 28°C for 48 hours (Hélias et al., 2012). Cavity forming single colonies on CVP plates were subcultured onto NA plates. Colony morphology of cavity-forming strains was compared with original cultures.

RESULTS and DISCUSSION

Among *Pectobacterium* species, *P. carotovorum* and *P. brasiliense* have larger geographical distribution with a broad host range spectrum, in contrast to *P. atrosepticum,* which is mostly restricted to potato and especially found more in cooler climates like European countries (Ma et al., 2007; Czajkowski et al., 2011). Recently, there have been reports about the presence of *P. polaris* causing blackleg and soft rot disease in potato,soft rot in bittersweet and Chinese cabbage plants. In addition to occurrence of *P. polaris* in several parts of the world, it was also detected for the first time in Turkey in 2019 (Oztürk, 2022).

It is aimed to determine the host range and consider the risk status of the native Turkish strains on plants belonging to different families. *P. polaris* strains caused disease symptoms in all the artificially inoculated plants. In general, wetness and weakening of tissue integration, paleness and discoloration of leaves were observed at the inoculation points. The invaded tissues by soft rot bacteria become soft and are transformed into a slimy mass consisting of numerous bacteria swimming about in the liquefied substances (Agrios, 2006). The formation of disease symptoms triggered by strains of *P. polaris* varied among inoculated plant species (Figure 1).

In particular, since cucumber, melon, squash and watermelon plants belonging to the Cucurbitaceae family have a more watery plant structure, which was more essential for the spread of the bacterial agent, faster disease development was noticed in the plant tissue of these plants. While there was almost no symptoms of yellowing in the plants, drying occurred after the formation of a mostly bacterial aqueous wet structure. In the following days, it was observed that the stem and leaves of tested plants became necrotic. As a result, wet-watery infection and plant collapse was first observed for these plants when compared to other plant species.

For lettuce plants, as a result of the progression of the bacterial agent within the vascular bundles from the point of application, first weakening of tissue integration in and around the main vein, and an increase in softening of the leaves, typical bacterial wetness with rotting in brown color and rot were observed 1-3 days after the application as a result of the bacterial agent moving towards the capillaries.

Disease symptoms like yellowing, drying, watery wet and plant collapse were observed more clearly in red cabbage, broccoli, and cauliflower plants in comparison to tomato, eggplant, and pepper. After 7-10 days, when the vascular bundles of the plants were cut and divided, it was observed that the bacterial rotting turned to systemic and progressed bidirectionally on the stem tissue, internal discharging from the inoculation point occurred, tissue thinning from the inoculation area in both directions, and then the stem was overturned from these regions. In the following days, wilting, drying, embrittlement, breakage, and wrinkling of the leaf stems were observed in the yellowing leaves. It was observed that the stem tissue gradually became thinner, and as a result, the broken stem felt down.

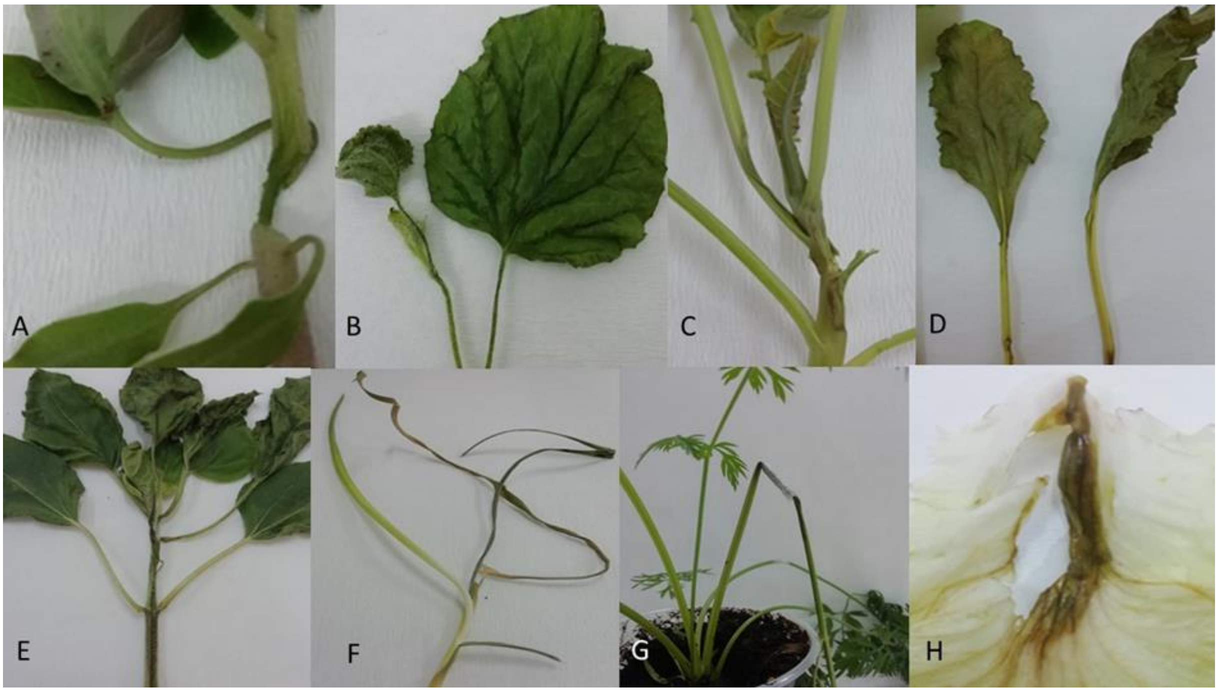

Figure 1. Soft rot disease symptoms (arrows) caused by *P. polaris* strain NV19 on artificially inoculated (A) tomato, (B) melon, (C) cauliflower, (D) sugar beet, (E) sunflower, (F) garlic, (G) carrot, and (H) lettuce plants

In the sunflower plants, 4-5 days after the inoculation, the bacterial agent progressed from the stem to the petioles, and specific bacterial wetness occurred on the main veins of the leaves. Later, deterioration and falling down from the inoculation point of stem tissue, formation of inky-brown tissue in the leaves, and wrinkled and dry leaves were observed in the following days. Similar observations were also recorded in the carrot plant, and in the same time intervals. In garlic and onion plants that were inoculated on leaves, yellowing, wrinkling, and necrosis were observed. Plant death was recorded in 14-15 days. Slow symptom development was observed for sugar beet plants inoculated from leaf petioles. Pale brown type necrosis on petioles turned completely brown-black, and wrinkled leaves dried and became like a gazelle after 14-20 days. This took a longer time in comparison to all other plants tested in this study.

Bacterial colonies were re-isolated successfully from symptomatic tissue of inoculated plants on modified CVP medium, and cavity-forming colonies represented the same colony growth with original cultures on NA plates with a transparent, gray color and crater colony morphology described as the typical colony morphology for *Pectobacterium* spp. by Ozturk et al. (2018).

The species of *Pectobacterium* which occur in potato-growing regions differs over time and geographical location. In the past, *P. carotovorum* (*Erwinia carotovora* pv. *carotovora*), *P. atrosepticum* (*Erwinia carotovora* pv. *atroseptica*), and *Dickeya* spp. (*E. chrysanthemi*) were the major soft rotting organisms. Today, more species in *Pectobacterium* spp. have been described (Portier et al., 2019) and it is estimated that the reason for wide geographical distribution and host range is the greater genetic variation among strains of *P. carotovorum* and *P. brasiliense,* both of which are the most threatening species of *Pectobacterium* genus with greater host range. Up to date, *P. carotovorum* has been reported on lettuce, garlic, pepper, eggplant, okra, leek, cabbage, chicory, squash, sugar beet, tomato, cucumber, celery, tobacco, mulberry, especially in temperate and semi-tropical regions (Gardan et al., 2003; Ma et al., 2007; Waleron et al., 2002; Waleron et al., 2014; Dadaşoğlu and Kotan, 2017; Öztürk and Soylu, 2022). Similar to *P. carotovorum*, *P. brasiliense* is known to be predominant and has been sporadically reported on potato all over the world, and on other crops such as lettuce, cucumber, carrot, and pepper (Mashavha, 2013; She et al., 2017; Soylu et al., 2022), cabbage and sugar beet (Waleron et al., 2015), squash (Zlatkovic et al., 2019), tomato (Caruso et al., 2016) and artichoke (Cariddi and Bubici, 2016) after its first occurrence in Brazil with severe blackleg infections on potato plants in 2004 (Duarte et al., 2004). For now, *P. polaris* has been reported in some parts of the world with a little infection ratio. The earlier determination of the host range of emerging pathogens is very important for risk status on other crops, and small minority can prove capable of causing major epidemics. For instance, *P. brasiliense* has been detected in many plant hosts in crop loss. If considered in this coverage,there is a possibility that *P. polaris* may be reported more in other plants and geographies in the future, which is now a very novel species of *Pectobacterium* taxa and was mostly reported not only on potato plants but also on Chinese cabbage and bittersweet for the present. Moreover, the most accelerating factor may be assumed as the seed potato tubers, which is the main source of bacterial dispersal for long distance. Especially latent infected propagation tuber material is so crucial for the transfer of pectinolytic pathogens (Czajkowski et al., 2011; Toth et al., 2011).

In concluion, all the defined host types are potential sources for *P. polaris*. The risk status of these plants, which have economic importance in Turkey, whose agricultural economy is the 8th largest in the world (Aytop et al., 2014), has been revealed in this study. Results demonstrated that pathogen is not only restricted to potato, but can also be pathogenic in the plants tested in this study. Therefore, demanding improved crop breeding, better disease management, and strict quarantine practices for controlling primarily potato blackleg disease should be carried out in the country to prevent introducing the pathogen to new areas.

ÖZET

Amaç: *Pectobacterium polaris,* dünyanın farklı bölgelerinde bildirilen ve yakın zamanda Türkiye'de tespit edilen *Pectobacterium* spp.'de yer alan yeni bir bakteriyel patojendir. Bu çalışmada *P. polaris*'in konukçu aralığını belirlemek için, patojenin iki ayrı izolatının farklı konukçularda patojenisitesini değerlendirmek ve etmene karşı risk altında olabilecek bitki türlerinin belirlenmesi hedeflenmiştir.

Yöntem ve Bulgular: Nevşehir ilinde karabacak hastalık belirtisi gösteren patates bitkilerinin enfekteli gövde dokusundan 2019 yılı vejetasyon döneminde izole edilmiş *P. polaris* (NV3 and NV19) izolatları bitkilere yapay inokulasyonlar için kullanılmıştır. İzolatların bakteriyel süspansiyonları hazırlanarak kırmızı lahana, brokoli, karnabahar, soğan, sarımsak, havuç, domates, biber, patlıcan, hıyar, kabak, kavun, karpuz, marul, ayçiçeği ve şeker pancarı bitkilerine bulaştırılmıştır. Yapay inokulasyon yapılan tüm bitkilerde meydana gelen hastalık simptomları ile *P. polaris*'in her iki izolatının hastalığa neden olduğu belirlenmiştir. Enfekteli bitki dokularından patojene ait koloniler yeniden izole edilmiştir.

Genel Yorum: *Pectobacterium* türleri, çok sayıda bitki türünde yumuşak çürüklüğe neden olan oldukça yıkıcı bakteriyel bitki patojenleridir. Dünyanın farklı bölgelerinde bildirilen ve ülkemizde oldukça yeni bir patojen olarak tanımlanan *P. polaris*'in geniş bir konukçu aralığında patojen olduğu belirlenmiştir. Patojenin ilk olarak izole edildiği bölgede hastalığın mücadelesine yönelik önlemler alınmalı ve özellikle patates yumruları yoluyla patojenin hastalıktan ari üretim alanlarına taşınması önlenmelidir.

Çalışmanın Önemi ve Etkisi: Testlenen tüm bitkilerin *P. polaris* için konukçu olduğu belirlenmiştir. Türkiye'de ekonomik öneme sahip olan bu kültür bitkilerinin patojene karşı risk durumları ilk kez bu çalışma ile belirlenmiştir.

Anahtar Kelimeler: Bakteriyel hastalık**,** ***Pectobacterium*** *polaris*, konukçu aralığı, patojenisite.

ACKNOWLEDGEMENTS

This study was partly supported by the financial grant of the project management office, Yozgat Bozok University, project no 6602c-ZF/21-472. The author would like to sincerely thank Prof. Dr. Soner SOYLU for valuable comments and important criticisms, and Ahmet DEMİRCİ for diligent proofreading of the manuscript.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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