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Original scientific paper

Pectobacterium carotovorum subsp. *carotovorum* - CAUSAL AGENT OF SOFT ROT OF PEPPERS PRODUCED IN THE STRUMICA REGION

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Abstract

Pectobacterium carotovorum subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*) is a plant pathogen that causes soft and stem rot diseases in several economically important vegetables such as carrot, cabbage, cucumber, eggplant, garlic, onion, pepper, potato, radish, sweet potato, squash and tomato, where the disease can be detected in the field, transmit, storage and market.

Agricultural producers face many challenges in trying to grow crops and ensure stable, high-quality yields. The risk factors involved in agricultural production include climatic conditions, the occurrence of diseases, pests, and weeds. During our field observation, we examined the production of pepper, its economic and nutritional value, and the factors contributing to its decline.

Our primary focus is the present status of diseases in pepper, specifically the occurrence of *Pectobacterium carotovorum* subsp. *carotovorum*, the causative agent of bacterial soft rot. *P. carotovorum* subsp. *carotovorum* is a well-known plant pathogen that causes severe soft rot disease in various crops, resulting in significant economic loss.

The aim of this study is to highlight the economic importance of the bacterial agent responsible for soft rot in peppers grown in the open fields in Strumica region. For this purpose, standard bacteriological tests were performed. These tests confirmed the pathogenic, morphological, biochemical, physiological, and growth properties of the pathogen, compared with the control strain KFB85 from the Republic of Serbia.

Key words: *diseases, pathogen, bacteriosis, plant bacteria, agricultural producing.*

INTRODUCTION

North Macedonia is a country located in the central part of the Balkan Peninsula, with ideal topographic and climatic conditions for agricultural production. Since ancient times, food production has been the primary means of livelihood in this country. Food production continues to be the subject of numerous and ongoing research worldwide.

Pepper (*Capsicum annuum* L.) is an annual, herbaceous plant of *Solanaceae* family. It originates from Brazil and it is one of the most widely cultivated crops in North Macedonia. Due to its nutritional value and distinct taste, this crop has become an indispensable part of the daily diet. Pepper is a vegetable rich in nutrients, carbohydrates, minerals, and vitamin C, with a concentration as high as 260 mg/100g. It also contains B vitamins and secondary metabolites, with capsaicin being the most abundant (Bosland & Votava, 2012).

In our country, these vegetables are produced both in open fields and greenhouses. The main agricultural regions in North Macedonia are Strumica, Gevgelija, Valandovo, Skopje, Bitola, and Kumanovo. The regions of Strumica, Gevgelija and Valandovo are particularly known for peppers, tomatoes and cucumbers production (Krsteska et al., 2022).

In 2019, the total arable area in North Macedonia was 519,452 ha, with 8,460 ha in the Strumica region, 8,730 ha in the Stip region, and 17,873 ha in the Skopje production region. Annually, 185,452 t of pepper are produced, with nearly 50% coming from the southeastern

region of the country. The most widely cultivated pepper types are kapija type, long (banana) type and different types of hot varieties (Statistical Office of the Republic of Macedonia, 2020).

The high demand and intensive production of pepper bring a series of risk factors, such as the occurrence of diseases, pests and weeds. The occurrence of plant diseases, specifically bacteriosis, is the subject of research in this paper.

Pectobacterium carotovorum subsp. *carotovorum* is the causal agent of bacterial soft rot in pepper and many other plants (Opara & Asuquo, 2016). Interest in studying this bacterium arose after the massive damage it caused to potatoes in Germany. The discovery of *Pectobacterium carotovorum* subsp. *carotovorum* occurred between 1878 and 1900 and it is regularly present in crops cultivated in North Macedonia (Mitrev, 2001; Pejcinovski & Mitrev, 2007).

Pectobacterium carotovorum subsp. *carotovorum* is a phytopathogenic, rod-shaped bacterium with dimensions of 0.5–0.8 × 1.3 mm. It is anaerobic and gram-negative. The arrangement of its flagella is peritrichous, indicating its high mobility. It does not form spores. When isolated on a nutrient medium, the bacterium forms shiny, round, smooth, gray-white colonies, noticeable after 24 hours of incubation (Jee et al., 2020; Lee et al., 2013).

Pectobacterium carotovorum subsp. *carotovorum* hydrolyzes gelatin and esculin, produces hydrogen sulfide, and has the ability to reduce nitrates. The bacterium is catalase-positive and oxidase-negative and produces phenylalanine deaminase, urease, arginine dehydrolase, lecithinase, and phosphatase. One of the most characteristic features of this pathogen is its ability to secrete pectolytic enzymes, which disorganize, soften, and lead to the complete decomposition of plant tissues (Charkowski, 2018; Gašić et al. 2014).

The bacteria typically enter plant tissue through wounds caused by using agrotechnical measures, rain, hail and insects. The most severe infections caused by *Pectobacterium carotovorum* subsp. *carotovorum* typically occur following hailstorms due to the extensive injuries they inflict (Arsenijević, 1988; Arsenijević & Obradović 1996; Ivanović et al., 2009).

MATERIAL AND METHODS

In July and August 2019, we collected symptomatic and asymptomatic pepper samples, from the open fields and greenhouses in Strumica region. The isolates were taken from pepper plants surveyed both in open fields and greenhouses (Figure 1).

Isolation of the pathogen was performed the same day as sample collection. The collected peppers were washed under tap water and then dried at room temperature. Next, small pieces from the symptomatic tissue (the margins between healthy and diseased tissue) were cut and homogenized with sterile deionized water (SDW). After waiting approximately for 5 minutes, the suspension was streaked onto Petri dishes containing nutrient agar (NA) and nutrient agar enriched with sucrose (NAS). The plates were incubated at 26°C for 48 hours.

Small, round, milky-white bacterial colonies that grew on the nutrient agar medium were selected as representatives. Fresh bacterial colonies were then used for further identification of the pathogen (Figure 2). The control strain KFB 85 from the Republic of Serbia was used for comparison. After bacterial colonies characteristic of the desired pathogen developed, some of them were selected for further analysis.

Five isolates, coded as Pcc 5, Pcc 7, Pcc 8, Pcc 13 and Pcc 20, were good types for future research from all collected samples.

Figure 1. Symptomatic pepper fruits collected from the Strumica region.

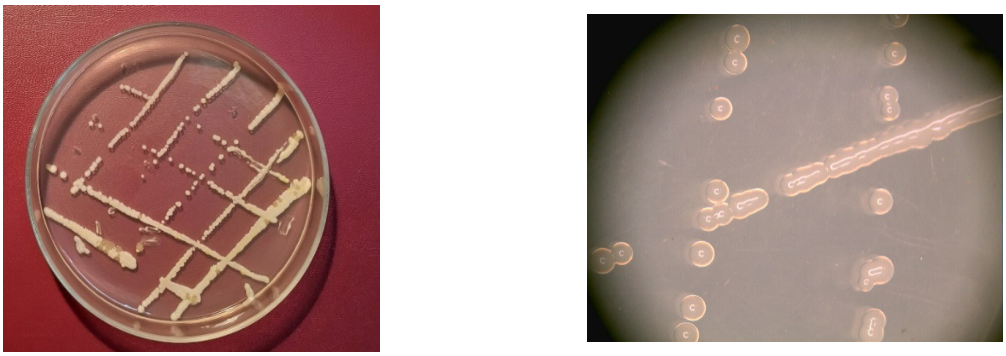

Figure 2. Pure bacterial colonies on NAS medium.

The main virulence determinants of soft-rotting *Pectobacterium* species are cell wall-degrading enzymes, primarily pectinases and cellulases. Identification of the strains was performed using bacteriological tests such as Gram's reaction, hypersensitivity on tobacco, tolerance to 5% and 7% NaCl, oxidase, catalase, pectolytic tests on potato and carrot, and hydrolysis of aesculin (EPPO Bulletin, 2023).

The ability to macerate the host tissue distinguishes this bacterium from many others. The pectolytic activity of the isolated *Pectobacterium* strains was assessed using potato and carrot fruit tissues (Figure 3). Potato tuber and carrot slices were washed with sterile deionized water (SDW), and holes with diameter of 60-70 mm were made. The holes were filled with fresh bacterial suspension (10^9 CFU/ml) and placed in sterile Petri dishes. Sterile distilled water was used as a negative control, while the KFB85 strain as positive control.

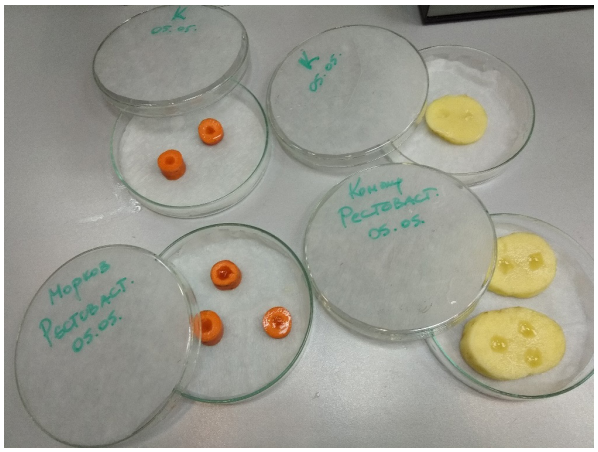

Figure 3. Assessment of pectolytic activity on potato tuber tissue and carrot root tissue.

Pectobacterium carotovorum subsp. *carotovorum* induces a hypersensitive response in tobacco plants. To confirm this, we performed a tobacco sensitivity test by injecting a bacterial suspension (10^9 CFU/ml) into the interveinal tissue of a tobacco leaf. The inoculation procedure is shown on Figure 3. The inoculated tobacco plant was incubated at 25-26°C, and the hypersensitive response was recorded after 24-48 hours (Figure 4).

Figure 4. Inoculated tobacco plant ready for incubation at 25-26°C in UNILAB.

RESULTS AND DISCUSSION

During July and August 2019, symptomatic pepper fruits were collected to confirm the presence of *Pectobacterium carotovorum* subsp. *carotovorum*. The symptomatic fruits exhibited watery tissue, mostly near the plant stem, and some produced an unpleasant odour. Bacteriological and pathogenic tests for our isolates were conducted and they were compared to the positive control strain KFB85. These tests confirmed the pathogen as *Pectobacterium carotovorum* subsp. *carotovorum*. This gram-negative bacterium is oxidase-negative, catalase-positive, and capable of hydrolysing aesculin. It demonstrates pectolytic activity on potato tuber and carrot root tissue and induces a hypersensitive reaction in tobacco leaves (Figure 5 and 6). Additionally, the bacterium demonstrates tolerance to 5% and 7% NaCl (Table 1).

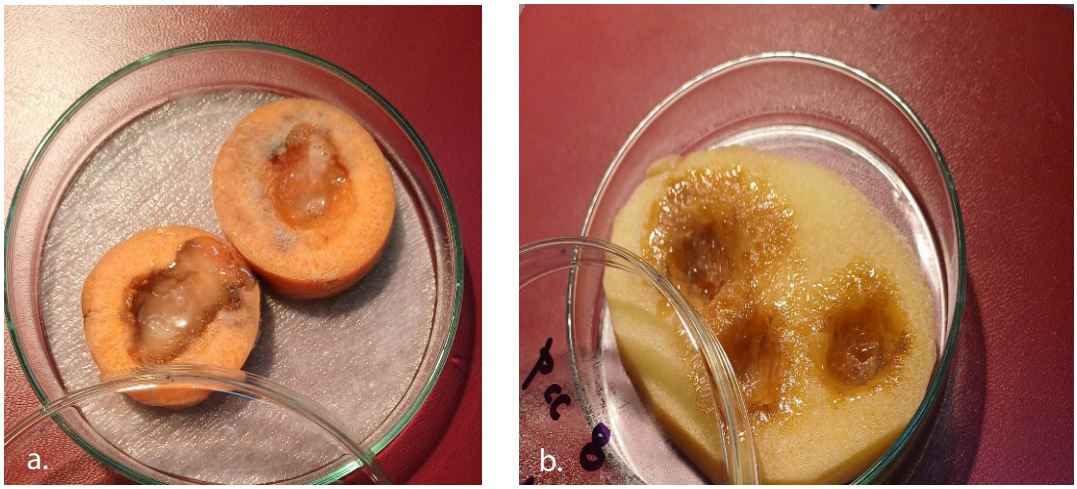

Figure 5. Results of bacterium pectolytic activity on a. potato tuber tissue b. carrot root tissue.

Figure 6. Hypersensitive response (HR) on tobacco leaves 48h after inoculation *Pectobacterium carotovorum* subsp. *carotovorum*.

Table 1. Biochemical and physiological characteristics of *Pectobacterium carotovorum* subsp. *carotovorum* strains and KFB85 as a control strain.

Properties	New isolated strains in UNILAB*					Control strain KFB 85 **
	Pcc 5	**Pcc 7**	**Pcc 9**	**Pcc 13**	**Pcc 20**	
Gram reaction	-	-	-	-	-	-
Activity of oxidase	-	-	-	-	-	-
Activity of catalase	+	+	+	+	+	+
Tolerance of 5% NaCl	+	+	+	+	+	+
Tolerance of 7% NaCl	+	+	+	+	+	+
Hypersensitive reaction (HR) on tobacco	+	+	+	+	+	+
Pectolytic activity on potato	+	+	+	+	+	+
Pectolytic activity on carrot	+	+	+	+	+	+
Hydrolysis of aesculin	+	+	+	+	+	+

+= positive reaction
- = negative reaction
*new isolated strains in UNILAB: Pcc5, Pcc7, Pcc9, Pcc13, Pcc20
**control strain KFB 85 (obtained by personal communication with A. Obradović, Republic of Serbia)

CONCLUDING REMARKS

The global demand for food requires intensive production, and science continues to advance in pursuit of this goal. The aim of this research is to emphasize the importance of constant monitoring to reduce the occurrence of phytopathogenic diseases. The detection and accurate identification of harmful plant pathogens are essential for improving plant disease control strategies (Kulukovska et al., 2021). Additionally, this study seeks to highlight the presence of *Pectobacterium carotovorum* subsp. *carotovorum* in our country.

It is important to note that *Pectobacterium carotovorum* subsp. *carotovorum* poses a serious threat to pepper production, particularly in the Strumica region, the largest vegetable production region in the Republic of North Macedonia, because the rapid spread of this bacterium leads to severe economic losses in the fields.

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Pectobacterium carotovorum SUBSP. *carotovorum* - ПРИЧИНИТЕЛ НА БАКТЕРИСКО ВЛАЖНО ГНИЕЊЕ КАЈ ПИПЕРКАТА ОДГЛЕДУВАНА ВО СТРУМИЧКИОТ РЕГИОН

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Резиме

Pectobacterium carotovorum subsp. *carotovorum* (порано познат како *Erwinia carotovora* subsp. *carotovora*) е растителен патоген кој предизвикува болести на влажно гниење на стеблото кај неколку економски значајни градинарски култури како: морков, зелка, краставица, модар патлиџан, лук, кромид, пиперка, компир, ротквица, сладок компир, тиква и домат, каде болеста може да се открие на терен, да се пренесе и да се складира.

Земјоделските производители се соочуваат со многу предизвици во обидот да ги одгледуваат земјоделските култури и да обезбедат стабилни, висококвалитетни приноси. Ризичните фактори вклучени во земјоделското производство ги вклучуваат климатските услови, појава на болести, штетници и плевели. За време на нашето теренско набљудување, предмет на истражување беше производството на пиперка, неговата економска и хранлива вредност и факторите кои придонесуваат за нејзиниот пад.

Нашиот примарен фокус е сегашниот статус на болести кај пиперката, особено појавата на *Pectobacterium carotovorum* subsp. *carotovorum*, предизвикувач на бактериско влажно гниење. *P. carotovorum* subsp. *carotovorum* е добро познат растителен патоген кој предизвикува сериозна болест на меко гниење кај различни култури, што резултира со значителна економска загуба.

Целта на оваа студија е да се истакне економската важност на патогенот одговорен за влажното гниење на плодовите пиперки одгледувани на отворено во Струмичкиот Регион. За таа цел беа направени стандардни бактериолошки тестови. Овие тестови ги потврдија патогените, морфолошките, биохемиските, физиолошките и својствата на раст на патогенот, во споредба со контролниот вид KFB85 од Република Србија.

Клучни зборови: *болести, патоген, бактериоза, растителни бактерии, земјоделско производство.*