Biotechnological Journal of Environmental Microorganisms (BJEM)

BJEM

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(4) 2022 177-185

Isolation and Characterization of the Symbiotic-Pathogenic Bacteria isolated from *Trifolium resupinatum* Plant

Nazanin Moazenpour[1], Monir Doudi*[2], Ladan Rahimzadeh Torabi [3], Mohammad Hossein Pazandeh [4]

[1] Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
[2] Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
[3] Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
[4]Department of Pharmacology and Toxicology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

Received: 9 December 2022/ Revised: 24 December 2022/ Accepted: 25 December 2022

Abstract

Plant diseases have a significant impact on plants and their crop yields, causing extensive epidemics and recurrent damages that result in profound negative effects. Bacteria such as *Erwinia, Pectobacterium* and *Klebsiella* have a very wide host range and can play a pathogenic role for a large number of ornamental and agricultural plants or even establish a symbiotic relationship with the plant. This group of bacteria that cause all kinds of plant diseases are able to affect seed tubers and soil microbial community. The objective of this investigation was to identify and classify the distinct symbiotic pathogens associated with *Trifolium resupinatum* plants obtained from the Shahreza region located in the southern part of Isfahan, Iran. In this investigation, *T. resupinatum* specimens harboring nodular root structures were initially identified and subsequently retrieved from various locations in the southern region of Isfahan (Shahreza), before being transported to the laboratory. The Yeast Mannitol Agar (YMA) medium underwent a cultivation procedure, subsequent to which the bacterial samples were subjected to molecular identification utilizing morphological and biochemical tests. Additionally, the colony-PCR technique was employed to achieve definitive identification. This study examined the molecular features of three distinct species namely *Erwinia chrysanthemi, Pectobacterium carotorum*, and *Klebsiella oxytoca*. It was revealed that the former two species exhibited a symbiotic pathogenic relationship with the *T. resupinatum* plant, while the latter species posed a threat to human health as a pathogen. The study results revealed that the root nodes of leguminous plants displayed the coexistence of beneficial symbiotic rhizobium species that are known for their capacity to enhance plant growth in both dicotyledonous and monocotyledonous plants.

Key words: Symbiotic-Pathogenic Bacteria, *Trifolium resupinatum*, *Erwinia chrysanthemi, Pectobacterium carotorum*, and *Klebsiella oxytoca*

*Corresponding Author: E-mail: monirdoudi@yahoo.com

Introduction

Pathogens are the causative agents of diseases in plants. Pathogens can be classified into two categories: living and non-living. The group of living pathogens encompasses fungi, viruses, bacteria, nematodes, viroids, protozoa, and parasitic flowering plants. Non-living pathogens, on the other hand, are responsible for causing non-infectious diseases in plants and can be attributed to environmental factors such as deficient food elements, extreme temperatures, air pollution, inadequate moisture levels, oxygen deprivation in the surrounding environment, poisoning from toxic elements, insecticides, fungicides, or herbicides, failure to adhere to agricultural principles, inappropriate soil pH, and imbalance in light exposure (Javaheri et al. 2022; Tabatabaei Ahmadrezaei et al. 2021; Yazdani et al. 2023). The *Pectobacterium* genus is a notably significant Gram-negative plant pathogen classified within the *Enterobacteriaceae* family. The members of this genus cause a wide range of disease symptoms, including soft rot and wilting, in both monocot and dicot host plants, as well as black leg in potatoes and certain shrubs (Marquez-villavicencio et al. 2011). The bacteria exhibit a specific trait where they actively manufacture and discharge enzymes that are capable of degrading the cell wall. Notably, pectinase or pectolytic acid enzymes are prevalently involved in this process, resulting in the disruption and fragmentation of plant tissues (Barras et al. 1994). The classification of the genus *Pectobacterium* is primarily determined by variations in host range as well as biochemical and molecular characteristics. This genus is further subdivided into multiple species and subspecies (Kwon et al. 1997). *P. atrosepticum* and *P. carotovorum* subsp. carotovorum (Pcc) species are particularly significant as they inflect severe harm on multiple agricultural cultivations in both field and storage conditions. Among these options, Pcc exhibits a broader spectrum of host compatibility (Zheo et al. 2010). *Erwinia chrysanthemi* bacteria, like other plant pathogens, are commonly present in agricultural products and pose a hidden risk. This bacterial pathogen is responsible for inducing soft rot disease on plants belonging to the Solanaceae family, both in field settings and during storage conditions. The existence of it has led to substantial agricultural damages, causing crop reductions of 20-40% in Isfahan province and even more severe effects across the country. In order to manage the presence of *Dickeya dadantii*, a bacterium currently referred to by this name, a range of interventions including copper compounds, ethylenediaminetetraacetic acid (EDTA), and diverse antibiotics are employed. Currently, phage therapy is emerging as a novel approach in agriculture for addressing the challenges associated with this particular pathogen (Korniienko et al. 2020). The critical significance of the vast epiphytic colony of this bacterium is indispensable in the progression of anthrax, owing to two separate factors. Firstly, it contributes to the initiation of infection within the floral system. Secondly, it facilitates the dissemination of bacteria among flowers through rain or pollinating insects, such as bees (Johnson et al. 1993). The colonization of the stigma surface by antagonist bacteria, prior to the arrival of pathogenic bacteria, may lead to an augment in their population. The critical significance of the vast epiphytic colony of this bacterium is indispensable in the progression of anthrax, owing to two separate factors. Firstly, it contributes to the initiation of infection within the floral system. Secondly, it facilitates the dissemination of bacteria among flowers through rain or pollinating insects, such as bees (Johnson et al. 1993). The colonization of the stigma surface by antagonist bacteria, prior to the arrival of pathogenic bacteria, may lead to an augment in their population (Pusey 1998). Legumes play a significant role as a vital source of protein, starch, oil, minerals, and vitamins. The seeds of this plant are highly valued in both human and livestock food industries due to their advantageous compounds, serving as a significant influence on the customary dietary practices of various populations worldwide. In terms of vegetarianism, it could be seen as a feasible substitute for traditional meat products (Schmidt and Viviani Ruffo 2023). The cultivation and adoption of improved and high-performing variants are crucial in the domain of leguminous fodder plants, especially concerning *T. resupinatum*. Re-

search indicates that indigenous populations of Iranian clover (*T. resupinatum*) exhibit substantial genetic diversity and possess the capacity for increased productivity, surpassing that of foreign cultivars. Thus, the refinement and purification of these populations holds the potential to generate enhanced and high-yielding strains. The findings of a comparative analysis between grass family fodder and alfalfa have indicated that alfalfa fodder exhibits low fiber content and high protein content, whereas grass fodder displays high fiber content and low protein content (Canale et al. 2002). The investigation into the influence of planting techniques and harvest timing on the nutritional value of Iranian clover fodder in Turkey revealed that early harvesting results in a deficiency of copper in the fodder. Additionally, the inclusion of Iranian clover in mixed crop systems has been shown to decrease the reliance on fertilizers for the companion plant while simultaneously enhancing both the quantitative and qualitative yield of fodder (Kokmaz 1993). *Klebsiella*, a gram-negative bacillus, is a member of the *Enterobacteriaceae* family, exhibiting a genetic affinity with several other genera within this family, including *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*. *Klebsiella* organisms exhibit a lack of motility and typically possess capsules. Certain sugars, namely lactose and sucrose, undergo fermentation. A significant number of strains typically exhibit the capacity to produce gas as a result of sugar fermentation, with the ability to generate gas from starch serving as a crucial aspect of diagnostic analysis (Murray et al. 2005). The unintended consequences of *Klebsiella oxytoca* on plants have been suggested by recent discoveries (Long et al. 2022). The significance of *Trifolium* cultivation is underscored by several factors, including its diverse species composition, ability to adapt to a wide range of climatic conditions, and high protein content in its aerial parts. However, research on the pathogens affecting this plant remains limited, necessitating further investigation. This study aimed to investigate and molecularly analyze specific symbiotic pathogens linked to *T. resupinatum* plants collected from the Shahreza area in the southern portion of Isfahan.

Materials and Methods

Plant collection

Shahreza is situated at a distance of 508 kilometers to the south of Tehran, and approximately 80 kilometers to the southwest of Isfahan. In order to carry out this research, first *T. resupinatum* plants with nodular roots from one of the southern regions of Isfahan (Shahrezah) which have the least restrictions in terms of soil EC and pH and also in terms of weather conditions (Fig 1). The growing conditions of this plant were suitable and they were collected at the right time (from March 20 th to April 20 th). Because the short flowering period of *T. resupinatum* in Isfahan province and especially in Shahreza region limited the sampling time, because on the one hand, due to the variety of species, the presence of flowers is necessary to identify the species of *T. resupinatum*. On the other hand, early heat in the early Spring causes the flowers to fall and the desired plants to almost dry up

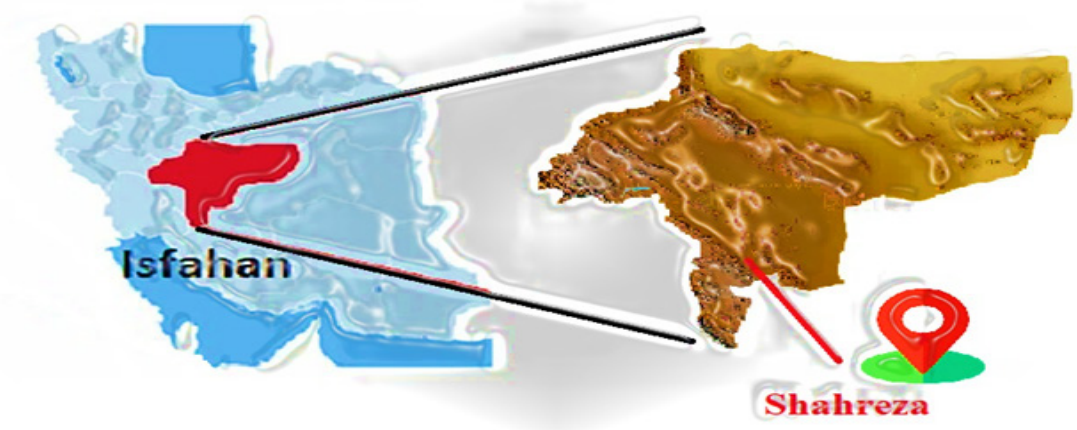

Fig.1. The geographical map of the Shahreza, Isfahan, Iran

Biotechnological Journal of Environmental Microorganisms (BJEM)

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(4) 2022 177-185

Fig.2. Root nodes of *T. resupinatum* plant in Shahreza region in the south of Isfahan

Separation of nodules

In the laboratory, first, the roots of the samples were washed with a gentle stream of water, and the aerial parts were transferred to the herbarium of the Islamic Azad University, Falavarjan branch, for the precise identification of *T. resupinatum* species (Fig 2).

Typically, the isolation of bacteria from nodules is considered a more straightforward and dependable approach compared to soil. Therefore, the bacteria were separated and recognized in controlled laboratory conditions, following the subsequent procedural series: The nodes were subjected to sterilization by being soaked in a solution of 95% ethanol and Hg_2Cl_2 for 5-10 seconds. In this experiment, aseptic knots were carefully transferred to a test tube that had 0.5 ml of sterile saline solution, which had a concentration of 8.5 g/L. To ensure sterility throughout the procedure, the knots were subsequently crushed with a sterile mortar. In this experiment, a total of 0.1 mL of node suspension and salt solution was carefully transferred onto the YMA culture medium, which had been prepared in a 9 cm plate. The spreading of the suspension onto the medium was facilitated using the assistance of Lupsteron. The plates were maintained in an incubator at a controlled temperature range of 25-28 °C. Subsequently, they were subjected to an incubation period lasting between 2 to 5 days. To assess the development of bacterial colonies along the culture lines, the plates were subsequently transferred onto alternative culture media, namely BHI and EMB. In order to guarantee the separation of untainted bacterial strains, a purification process consisting of multiple stages was carried out. Different colonies with distinct appearances were cultured on individual plates. The investigation involved analyzing the heat resistance test and growth rate of the isolates, while gathering diverse information about them using conventional phenotypic identification methods (Shafizadeh et al. 2016). After the biochemical identification of the isolates, the researchers proceeded to prepare the desired samples for testing Molecular models (Sangeetha et al. 2020; Vasundhara and Thammaiah 2017).

Table 1: Sequences of the general primers in this study (Moazzenpour et al. 2018)

Primers	Length of primer	Sequence (3’ 5’)
OF BUN	18 N	CGCATTTCACCGCTACAC
OR BUN	18 N	TATGTACACACCGCCCGT
IF BUN	18 N	TAAACCACATGCTCCACC
IR BUN	18 N	ACACACGTGCTACAATGG

Table 2: The thermal cycle used to perform the colony-PCR technique in this study

PCR step	Time (Sec)	Temperature (°C)	Number of cycle
Initial denaturation	300	94	۱
Denaturation	45	94	۳۵
Annealing	45	57	
Extension	45	72	
Final extension	300	72	۱

Colony-PCR method

Colony-PCR is a method used to confirm the existence of a specific gene fragment in bacteria. During the investigation, we used general primers that were specifically made for the 16S ribosomal DNA (rDNA) gene, more details of which can be found in Table 1. Talley Gene and Sina-Clone Company in Iran carried out the complete process of conceptualization and production of these primers (Karami et al. 2022).

In this specific method, a small portion of the purified colony was mixed with 10 µL of sterile distilled water using a sterilized loop, and then used as a template for the polymerase chain reaction (PCR) process. Table 2 illustrated the requisite values and conditions essential for carrying out the colony-PCR methodology.

Quality control of PCR products using gel electrophoresis

Agarose gel electrophoresis was used to assess the quality of PCR products. The velocity of DNA migration within the gel exhibits a linear relationship with the applied electric field intensity. The process of electrophoresis starts when an electric current is introduced, causing DNA to migrate towards the positive pole as a result of its negative charge. The velocity of this movement is governed by the DNA molecule size and the concentration of agarose present in the medium. Ultimately, the gel was subjected to ultraviolet irradiation using the gel dock equipment, resulting in the identification of the targeted band. The manifestation of a solitary intense band within the designated range serves as a reliable indicator of successful polymerase chain reaction (PCR) amplification.

Results

In this research, *T. resupinatum* plant with nodular roots was identified from the agricultural soils of Shahreza city, located in the south of Isfahan province, and was transferred to the laboratory. According to the diagnosis of the herbarium specialist of Islamic Azad University, Falavarjan Branch, the largest number of *T. resupinatum* samples collected was related *to Trifolium tomentosa* species. The phenotypic diagnosis of *T. resupinatum* plant parasitic bacteria was based on morphological diagnosis and gram staining, the bacteria isolated in YMA, EMB, BHI culture media created wormy and slimy colonies and their gram reaction was negative and had an aggregated arrangement and under the microscope All bacteria were observed as rods according to Figure 3.

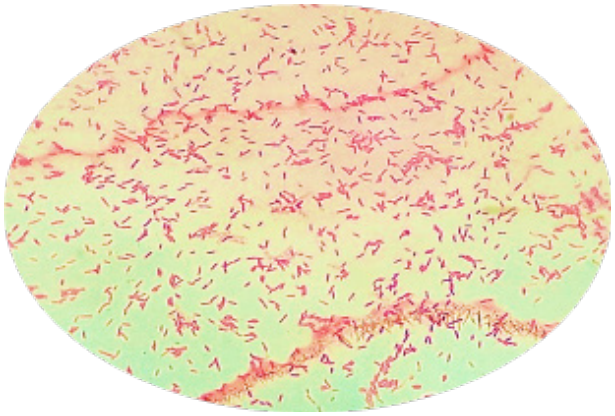

Fig. 3. Microscopic view of one of the pathogenic bacteria isolated from *T. resupinatum* plant nodes with the help of gram staining

The results of biochemical tests for bacteria isolated from *T. resupinatum* plant nodules included the following: *Erwinia chrysanthemum*: (oxidase negative, catalase positive, indole positive, phosphatase positive, gelatinase negative, sensitive to the antibiotic erythromycin), *Pectobacterium carutoverum*: (oxidase negative, catalase positive, OF glucose positive, gelatinase positive, methyl red negative) and *Klebsiella oxytoca*: (indole positive, oxidase negative, citrate positive, urea hydrolysis positive, lysine decarboxylase positive, methyl red negative and

Vege-Prosquare positive). After performing colony-PCR, bacteria from three genera *Erwinia chrysanthemi, Pectobacterium carotorum*, and *Klebsiella oxytoca* were detected according to the gel image in Figure 4.

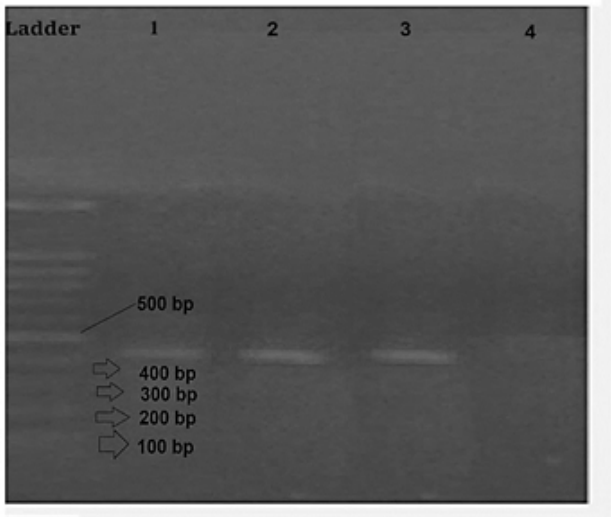

Fig. 4. Results of electrophoresis of PCR product, bacterial strains of *T. resupinatum* plant pathogen isolated with universal primers on 1% agarose gel, in this figure, 100 bp weight marker was used. Line L: the weight Ladder, line 1: *Erwinia chrysanthemi* strain (430 bp), line 2: *Pectobacterium* carotorum strain (420 bp), line 3: *Klebsiella oxytoca* strain were 420 bp, line 4: negative control (distilled water).

This study successfully identified and characterized three distinct genera (*E. chrysanthemi*, *P. carotorum*, and *K. oxytoca*) from the tubers of the *T. resupinatum* plant, often found in association with the renowned species *Trifolium*. The identification of the plant samples was carried out through a meticulous examination of the herbarium records at Islamic Azad University, specifically the Falavarjan branch. The study mentioned above has shown that some species in the *Erwinia* genus have different roles as plant pathogens and can also help with plant nitrogen fixation through a symbiotic relationship (Vasundhara and Thammaiah 2017). However, it was observed in this study that the *Erwinia chrysanthemi* species is likely the causative agent of *T. resupinatum* plants in the Shahreza region, which is situated in the southern area of Isfahan city. According to local farmers' opinions, it seems that the places where this bacterium was discovered in the root nodules of *T. resupinatum* plants had worse conditions for plant growth compared to other areas. Therefore, the economic success of farmers operating in the specified area has suffered as a consequence of this situation. The researchers studied the taxonomy and general biology of *Xenorhabdus* and *Photorhabdus spp*., serving as the central topic of investigation. These bacteria's unique symbiotic and pathogenic life cycle has been explored using molecular biological techniques. The heightened pathogenic nature exhibited by *Xenorhabdus* and *Photorhabdus spp*. to cause infection in a susceptible host. The effectiveness and power of bacterial cells are evident in their ability to successfully target and enter desired hosts. In order to promptly inhibit the propagation and remove the infested larvae. Bacterial septicemia does not seem to significantly contribute to the insect's mortality (Forst and Nealson 1996). Despite the vast diversity of plant-microbe interactions, they follow a historical progression, forming a continuum of symbiotic relationships. Arbuscular mycorrhiza (AM) is the ancestral form that helped plants colonize land during the early stages of terrestrial flora evolution. During AM evolution, the plant acquired genes to regulate microbe colonization of root tissues. Later, genes were rearranged to accommodate new symbionts (N_2-fixing bacteria, ectomycorrhizal fungi, endophytes, and epiphytes) and pathogens in symbiotic interactions. From the microbial side, the evolution of mutualism and antagonism is limited to symbioses between plants and ergot fungi, *Clavibacter, Bacillus*, and *Pseudomonas* bacteria. Similar systems in symbiotic interactions may be related to convergent evolution in distant microorganisms (adaptation to host defense/regulatory factors), molecular mimicry (imitation of interaction mechanisms used by ancient symbionts), or horizontal gene transfer (Provorov 2009). Furthermore, *P. carotorum* has been discovered and documented on numerous ornamental plants as well as monocotyledonous and dicotyledonous plants across various regions of Iran. Unfortunately, this specific species is acknowledged as a plant pathogen, causing a substantial decrease in plant growth within the investigated regions (Baghaee-Ravari et al. 2011;Dahaghin and Shams-bakhsh, 2014). A study was carried out by Chung et al, in which they collected *Enterobacter*, *Klebsiella*,

and *Pantybacillus* bacteria from the rhizosphere of onion, pepper, and rice plants. They recognized that these bacteria have pathogenic qualities that go beyond affecting humans, as they can also function as opportunistic pathogens for the development of diseases in grass and pasture vegetation. The aforementioned plants are susceptible to soft rot, thereby leading to their deterioration. In the current investigation, the researchers have successfully isolated and molecularly identified *Klebsiella oxytoca* bacteria from the tuber or root nodule of *T. resupinatum* plants in the Shahreza region. This discovery suggests a notable similarity to prior studies carried out by these researchers. As part of the present investigation, scholars have managed to isolate and molecularly analyze *K. oxytoca* bacteria extracted from the tuber or root nodule of *T. resupinatum* plants in the Shahreza area. This finding bears considerable resemblance to the researchers' own inquiry. Several species of diazotrophic bacteria, namely *Erwinia, Azotobacter*, *Agrobacterium*, *Burkholderia*, and *Pseudomonas*, have the capability to enhance the uptake of phosphate and fix nitrogen in a bioavailable form. The augmentation of phosphorus bioavailability is achieved by microorganisms through the synthesis of organic acids, thereby facilitating the absorption of inorganic phosphorus (Scevino et al. 2010). Meanwhile, researchers' research shows that bacterial pathogens of some pasture plants sometimes lead to the loss of essential plant elements such as nitrogen, phosphorus and iron, even to the limited extent of microelements such as molybdenum, copper and zinc (Sangeetha et al., 2020; Vasundhara and Thammaiah 2017). The species of P. carotorum has been reported as a pathogenic agent from the plant families of Cabbage, Solanaceae, Umbrellas, Lilies, and Cucurbitaceae (Toth et al. 2003) and Arase (Hu et al. 2008), Spathiphyllum (Alipi and Lopez, 2009), Bromelia, Credlin, Syngonium, Aloe, Cereus, various species of cactus, Sedum (Chase 1997), tulip (Boyraz et al. 2006) and Euphorbia (Suslow and McKean 1979) has been isolated and reported from different regions of the world. The present study also uncovered evidence showing that the infected bacterium considerably hindered the growth of *T. resupinatum* plant tubers. Sinorhizobium strains have the capability to establish a symbiotic relationship with various plant species, including clover, alfalfa, and soybean, leading to their coexistence within the root systems. Furthermore, they actively engage in the biological process of nitrogen fixation, whereby atmospheric nitrogen is converted into a biologically usable form, contributing to the plants' nitrogen requirements. The use of rhizobial biological fertilizers in modern agriculture has become widely recognized as a way to increase the crop productivity of leguminous plants. Practitioners and experts increasingly prefer this approach due to its noticeable economic benefits as well as its positive impact on the environment (Panahpour 2007; Peoples et al. 1995). The current research being conducted in the south of Isfahan is motivated by a lack of previous studies on symbiotic bacterial pathogens linked to *T. resupinatum*. Furthermore, the assessment of economic losses and environmental health hazards stemming from such pathogens has remained unexplored. Future benefits are expected in relation to the future potential of offering practical solutions for farmers in the mentioned area and *T. resupinatum* growers in various regions. The objective of these solutions is to improve the methods of cultivating the plant and ensure a plentiful harvest, all while safeguarding its quality and overall well-being.

Conclusion

The research findings revealed that the root nodes of leguminous plants, such as T. resupinatum, exhibited the presence of not only beneficial symbiotic rhizobium species known to enhance plant growth in dicotyledonous and monocotyledonous plants, but also the coexistence of potentially harmful pathogenic bacteria. The presence of these plants' roots, accordingly leading to significant agricultural losses for farmers in the region. It is anticipated that the elimination and management of this particular pest may be achieved through the utilization of bacteriophages or plant-based pesticides in subsequent periods.

Acknowledgments

The present authors feel obliged to acknowl-

edge and extend their appreciation to Mrs. Engineer Shahsar and Parsafar, esteemed officials of the Islamic Azad University Research Laboratory, Falavarjan Branch, as well as to the official in charge of the plant herbarium at the same university, Dr. Khattabakhsh.

References

Alippi AM, Lopez AC (2009) First Report of *Pectobacterium carotovorum* subsp. *carotovorum* on *Spathiphyllum* wallisii in Argentina. Plant Disease. 93: 842-843.

Baghaee-Ravari S, Rahimian,H, Shams-Bakhsh M, Lopez-Solanilla E, AntúnezLamas M, Rodríguez-Palenzuela P (2011) Characterization of *Pectobacterium* species from Iran using biochemical and molecular methods. European Journal of Plant Pathology. 129: 413-425.

Barras F, Van Gijsegem F, Chatterjee AK (1994) Extracellular enzymes and pathogenesis of soft-rot Erwinia. Annual Review of Phytopathology. 32: 201-34.

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

Canale CJ،Glenn BP,Reeves JB (2002) Alkali-treated alfalfa and switchgrass:Composition and in situ disappearance of OM ,NDF and ADF, monosacharides. Dairy Sci. 85 :3411- 3419.

Cassida KA, Griffin TS, Rodriguez J, Hesterman OB, Rust SR (2000) Protein degradability and forage quality in maturing alfalfa, red clover and birdsfoot trifoli. Crop Sci. 40: 209- 215.

Chao YC, Feng CT, Ho WC (2006) First report of Aglaonema bacterial blight caused by *Erwinia chrysanthemi* in Taiwan. Plant Disease. 90: 1358. 12.

Chase AR (1997) Foliage plant diseases: Diagnosis and control, American Phytopathological Society Press. St. Paul, Minnesota, USA. 218.

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Chob H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol. Biochem. 37: 1970-1974.

Dahaghin L, Shams-bakhsh M (2014) Identification and genetic diversity of pectolytic phytopathogenic bacteria of mono- and dicotyledonous ornamental plants in Iran. Journal of Plant Pathology. 96: 271-279.

Duarte V, De Boer SH, Ward LJ, de Oliveira AMR (2004) Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. Journal of Applied Microbiology. 96: 535-545.

Forst S, Nealson K (1996) Molecular biology of the symbiotic-pathogenic bacteria *Xenorhabdus spp*. and *Photorhabdus spp*. Microbiol Rev. 60(1):21-43. doi: 10.1128/mr.60.1.21-43.1996.

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

Hu XF, Ying FX, Gao YY, Chen HM, Chen JS (2008) Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* causing soft-rot disease on Pinellia ternate in China. European Journal of Plant Pathology. 120: 305-310.

Javaheri G, Doudi M, Rahimzadeh Torabi L, Pazandeh MH (2022) *Nicotiana plumbaginifolia* Leaf Extracts as an Efficient Antibacterial and a Biocontrol Agent Versus Tomato Pathogenic Bacteria. Journal of Medicinal plants and By-product. 11(Special): 87-93. doi: 10.22092/jmpb.2020.352012.1261.

Johnson KB, Stockwell VO, McLaughlin RJ, Sugar D, Loper JE, Roberts RG (1993) Effect of antagonistic bacteria on establishment of honey bee dispersed *Erwinia amylovora* in pear blossoms and on fire blight control. Phytopathology. 83: 995-1002.

Karami E, Doudi M, Rezayatmand Z, Rahimzadeh Torabi L (2022) 'The Impact of Rhizospheric *Pseudomonas aeruginosa* on the Growth of *Melissa officinalis*', Biotechnological Journal of Environmental Microbiology. 1(3).

Kokmaz A, Gulser MG, Manga L, Sancak C (1993) effect of cropping system and cutting date for various forage crops on the mineral content and quality of hay produced in Samsun province. Doga Turk Torimve Ormancilik Dergisi. 17: 1069 -1080.

Korniienko N, Kharina A, Budzanivska I, Burketová1 L, Kalachova T (2020) Phages of phytopathogenic bacteria: High potential, but challenging application. Plant Protection Science.58(2):81-91.

Kwon SW, Go SJ, Kang HW, Ryu JC, Jo JK (1997) Phylogenetic analysis of *Erwinia* species based on 16S rRNA gene sequences. International Journal of Systematic Bacteriology. 47: 1061-1067.

Long H, Hu Y, Feng Y, Zong Z (2022) Genome analysis of *Klebsiella oxytoca* complex for *antimicrobial* resistance and virulence genes. *Antimicrob. Agents Chemother*. 66: e0218321. doi: 10.1128/aac.02183-21

Marquez-Villavicencio MDP, Groves, RL, Charkowski AO (2011) Soft rot disease severity is affected by potato physiology and *Pectobacterium* taxa. Plant Disease. 95: 232-241.

Moazzenpour N, Yahyaabadi S, Doudi M (2018) Three-Rhizobia Screening On the amount of photosynthetic pigments of The Cowpea (Vigna Unguiculata) Plant Which Was Under Nickel Stress. NCMBJ.9 (33) :49-60

Murray PR, Holmes B, Aucken HM (2005) *citrobacter*, *klebsiella*, *Enterobacter*, *serratia*, and other Enterobacteriaceae, in: Boriello SP, Murray PR, Funke G. Topley & Wilson's.10th Edition. London: Hodder Arnold.1474 -1506.

Panahpour H (2007) The effect of nitrogen fixing bacteria (*Sinorhizobium meliloti sp*.) in different regions on nitrogen fixing and alfalfa function in three varieties of alfalfa (*M. sativa L*.). Journal of Rangelands Forests Plant Breeding and Genetic Research.15:243 -252.

Peoples MB, Herridge DF, Ladha JK (1995) Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. Plant Soil. 174:3-28.

Provorov NA (2009) Plant-microbe symbioses as an evolutionary continuum. Zh Obshch Biol. 70(1):10-34.

Pusey PL (1998) Laboratory and field trials with selected microorganisms as biocontrol agents for fire blight. Acta Horticulture. 489: 655-660.

Sangeetha V, Harish S, Thiruvudainambi S, Beaulah A (2020) Phenotypic and Biochemical Characterization of *Pectobacterium carotovorum* sub sp *carotovorum*, the Incitant of Onion Soft Rot. International Journal of Current Microbiology and Applied Sciences. 9(12): 8-15.

Scervino JM, Mesa M P, Míonica ID, Recchi M,Moreno NS, Godeas A (2010) Soil fungal isolates produce different organic acid patterns involved in phosphate salts solubilization. Biol. Fertil. Soils. 46,755-763.

Schmidt HDO, Oliveira VRD (2023) Overview of the Incorporation of Legumes into New Food Options: An Approach on Versatility, Nutritional, Technological, and Sensory Quality. Foods. 12(13):2586.

Suslow T, McCain AH (1979) Etiology, host range and control of a soft rot bacterium from cactus. Phytopathology. 69: 921.927.

Tabatabaei Ahmadrezaei M, Pazandeh MH, Rahimzadeh Torabi L, Doudi M (2021) *Trachyspermum copticum* essential oil: an effective herbal and natural antimicrobial agent against human skin pathogenic bacteria. International Journal of Bio-Inorganic Hybrid Nanomaterials. 10(4): 193-205.

Toth IK, Bell K S, Holeva MC, Birch PRJ (2003) Soft rot *Erwiniae*: From genes to genomes. Molecular Plant Pathology. 4:17-30.

Van der Merwe JJ, Coutinho TA, Korsten L, Van der Waals E (2010) *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg on potatoes in South Africa. European Journal of Plant Pathology. 126: 175-185.

Vasundhara BA and Thammaiah N (2017) Morphological and Biochemical Properties Useful for Identification of *Erwinia chrysanthemi*. International Journal of Current Microbiology and Applied Sciences. 6(10): 343-348.

Yazdani S, Doudi M, Rezayatmand Z, Rahimzadeh Torabi L (2023) Investigating the antibacterial and phytochemical effect of methanol and acetone extracts of the *Cupressus sempervirens* and *Juniperus excelsa* on some important foodborne diseases. Journal of Medicinal Herbs, "J. Med Herb" (Formerly known as Journal of Herbal Drugs or J. Herb Drug). 14(1): 63-72. doi: 10.30495/medherb.2023.703159.

Zhu L, Xie H, Chen S, Ma R (2010) Rapid Isolation, identification and phylogenetic analysis of Pectobacerium carotovorum. Journal of Plant Pathology. 92: 479-486.