Bacterial flora of infected vegetable crops

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Abstract. The focal point of the article revolves around the examination of bacteria isolated from infected vegetable crops. The study encompasses a comprehensive analysis of various attributes including colony structure, color, and size of these isolated bacteria in their pure form. To enhance our understanding, fixed preparations were meticulously crafted from these isolates, followed by Gram staining. The isolates were scrutinized under immersion oil using an x 100 lens and specialized microscopy equipment, namely the xsp-136 B and N-300m microscope (UCMOSO9000KPB), magnifying them up to 1000 times. To further delve into the taxonomic classification of these isolated strains, the MALDI-TOF method was employed. The strains were precisely identified through this method, carried out at the Department of Sanitary and Epidemiological Control, utilizing state-of-the-art Bruker equipment. Central to the research was the exploration of the pathogenic properties of these bacteria in relation to plants. To this end, a pivotal experiment was conducted wherein seeds of tomatoes and eggplants were artificially planted using these isolated bacteria. The culmination of these investigations has resulted in the creation of four distinct tables, meticulously supplemented with figures to visually encapsulate the findings. In essence, the article presents a comprehensive exploration of the isolated bacteria's attributes, classification, and their potential pathogenic impact on plants.
Keywords. Bacteria, vegetable, tomato, eggplant, plant, root, stem, seed, leaf, fruit.

1 Introduction

Vegetable products are of great importance in human life and occupy an important place in the diet. One of the main conditions for ensuring a high and high-quality harvest in vegetable growing is to protect it from various diseases [1]. Vegetable crops such as tomatoes, peppers, eggplants and potatoes are damaged by several diseases during the growing season, which negatively affects the development of the plant, causing a sharp decrease in yield [2].

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At least 54 phytopathogenic fungi, 39 types of viruses, 19 types of nematodes, 3 types of phytoplasmas and 11 types of bacteria cause significant economic damage to the potato crop. Pathogenic bacteria cause various etiological diseases, from mild rot to "zebra chips" [3]. Worldwide, about 10,000 fungi from phytopathogenic objects, about 630 viruses, 200 bacterial species are responsible for the death of the tomato crop due to cassality [4]. Diseases caused by microscopic fungi (64 species), oomycetes (11), bacteria (12), phytoplasmas (3), viruses (32) and nematodes (B species) have been reported in pepper varieties worldwide [5].

In Uzbekistan, diseases of sweet pepper crops are mainly caused by fungi, bacteria and viruses. Pepper crops are mainly affected by black root rot, fusarium, late blight, cladosporiosis, gray and white rot, black bacterial spotting and tobacco mosaic diseases [6]. A number of bacterial diseases affecting various organs of the tomato crop have been detected: necrosis of the stem core, bacterial leaf lesions, fruit lesions, terminal rot of fruits, watery rot of stems and fruits, bacterial wilting of the stem (brown wilting), root diseases of cancer [7].

Phytopathogenic microorganisms affecting agricultural crops also include secondary pathogens, which can mainly populate the plant in places of mechanical damage and cause symptoms of various manifestations of the disease in the plant. But these microorganisms are not considered the main causative agent of plant disease [8]. In vegetable crops, the main pathogens damage the plant due to their phytotoxic properties. Therefore, it is very important to study the phytotoxic nature of microorganisms in order to determine whether they are pathogenic species in relation to the plant [9].

Currently, for the cultivation of tomato products of export quality, not only for the domestic market of our country, one of the most urgent tasks is to protect it from diseases and prevent morbidity. To do this, it is very important to identify phytopathogenic microorganisms that provoke the disease, identify their types and develop biological control measures against it [10].

The aim of the study is to identify the bacterial flora of infected vegetable crops and to study the properties of phytotoxicity of bacteria that have been scratched.

2 Materials and methods

Infected stalks and tomato leaves, sweet pepper leaves, stalks and fruits, potato leaves were delivered to the laboratory in separate packages. The imported samples were placed in a refrigerator at a temperature of 4 °C and processed to remove the external microflora within 48 hours.

The affected leaf, stem and root parts of plants were cut and washed in running water, then treated with a 3% solution of hydrogen peroxide for 2-3 minutes, and then rinsed again with sterile tap water. Dried on sterile filter paper, cut from pieces containing leaves, stems, damaged and intact healthy tissues, 1-3 cm in size and placed in Petri dishes containing GPA (meat-peptone agar) nutrient medium [3]. In the same way, the parts of plants sterilized from the outside were crushed in separate sterile porcelain baths, and the crushed samples were planted in GPA nutrient media, Petri dishes. The samples planted in the culture medium were incubated in a thermostat at a temperature of 27-28 ° C. Bacterial growth was observed within 24-48 hours.

Differentiated colonies of grown bacteria according to their appearance, color and morphology were selected and transplanted back into the GPA culture medium using a bacterial loop. To purify the isolated strains, this process was carried out 3 times [9]. The structure, color, and size of a colony of bacteria isolated in pure form were studied. Fixed preparations were also made from isolates and stained by gram method, examined under

immersion oil in an x 100 lens, an xsp-136 B and N-300m microscope (UCMOSO9000KPB) (magnified 1000 times).

The isolated types of bacteria were identified by the MALDI-TOF method in the Department of Sanitary and Epidemiological Control of the Main Department of Medicine under the Administration of the President of the Republic of Uzbekistan, using Bruker equipment.

When determining the type of bacteria, the main research methods remain a simple culture method and standard biochemical tests. The MALDI-TOF MS method has been used for almost 20 years mainly in the chemical laboratory for the identification of various molecules, such as sugars, nucleic acids and proteins [9, 10]. Currently, this method is used as an identification method in microbiological laboratories [2-5]. The systematic role of isolated bacteria in Bergi was determined [7, 8]. Bacteria GPB (meat peptone broth) were grown in a nutrient medium for 6 days. The cultural fluid was filtered.

To study the biological effects of phytotoxins on plant seeds, tomato of the “Sitora” variety and eggplant of the “Diamond” variety were ivitized into the fungal cultural fluid for 24 hours. 5 large, (healthy seeds) were used for each option. For control, nutrient media of non-fungal growth, sterilized liquid Chapek and GPB were applied.

For 24 hours, the ivitized seeds were put into collection in wet chambers in a petri saucer. The nature of the fungus to produce phytotoxins was determined by a decrease in the germination capacity of the seed, lagging behind the growth of the grass and root. Among the phytotoxin-forming species, 30% of the control introduced species that reduce the germination capacity of the seed, leaving its growth behind [1].

3 Results and discussion

In total, 15 bacterial isolates were isolated from samples brought from infected parts of potato, tomato and sweet pepper crops from Kasbi, Kashkadarya region and Shakhrisabz district, Upper Chirchik and Kibray district of Tashkent region, Bagdad district of Ferghana region (Table 1).

Table 1. Bacteria isolated from infected vegetable crops in Kashkadarya, Tashkent and Ferghana regions, Uzbekistan.

#	Isolates	Variety of agricultural crops	Plant organ	Distinguish between the name and cancers
1	Kasbi district, Qurbonov Rajabpo’lat Qurbonovich farm	Rossiya	Potato leaf	*Pseudomonas putida* 4/23
2	Kasbiv district, Qurbonov Rajabpo’lat Qurbonovich farm	Rossiya	Potato leaf	*Bacteroides ovatus* 4/22
3	Shaxrisabz district Davronov farm issiqxona	Vidrona	Sweet Pepper leaf	*Klibsiella oxytoca* 6/26
4	Kasbiv district, Qurbonov Rajabpo’lat Qurbonovich farm	Rossiya	Potato leaf	*Pseudomonas mendocina* 4/27
5	Юқори Чирчиқ district Жума бозор худуди Ф.Омонов farm	Yusupov	Tomato stalk	*Stenotrophomonas maltophilia* 2/16
6	Yuqori Chirchiq district Juma market F.Omonov farm	Yusupov	Tomato stalk	*Pseudomonas alcaliphila* 2/18
7	Yuqori Chirchiq district Juma market F.Omonov farm	Yusupov	Tomato stalk	*Stenotrophomonas maltophilia* 2/19
8	Yuqori Chirchiq district Juma market F.Omonov farm	Yusupov	Tomato stalk	*Brevundimonas diminuta* 2/20

9	Qibray district TashGRES "Mashxura Fayz Baraka" MChJ greenhouse with a view of	Pink Paradayz	tomato	*Stenotrophomonas* sp. 2/13
10	Qibray district TashGRES "Mashxura Fayz Baraka" MChJ greenhouse with a view of	Pink Paradayz	leaf tomato	*Pseudomonas Antarctica* 2/9
11	Qibray district TashGRES "Mashxura Fayz Baraka" MChJ greenhouse with a view of	Pink Paradayz	leaf tomato	*Stenotrophomonas* sp. 2/10
12	Qibray district TashGRES "Mashxura Fayz Baraka" MChJ greenhouse with a view of	Pink Paradayz	stalk tomato stalk	*Paenarhrobacter ilicis* 2/12
13	Qibray district TashGRES "Mashxura Fayz Baraka" MChJ greenhouse with a view of	Pink Paradayz	tomato stalk	*Microbacterium phyllosphaerae* 2/15
14	Qibray district TashGRES "Mashxura Fayz Baraka" MChJ greenhouse with a view of	Pink Paradayz	Tomato stalk	*Sphingobacterium faecium* 2/14
15	Farg'ona province Bog'dod district farm	-	Sweet Pepper leaf	*Microbacterium paraoxydans* 3/14

The morphology of the isolated bacteria was studied. The table and figures on sheep show colonies of bacteria isolated from the stems and leaves of Cypress, tomatoes brought from the tashgres greenhouse (Figure 1), microscopic appearance (Figure 2) and micromorphology (Table 2) during gramstraining.

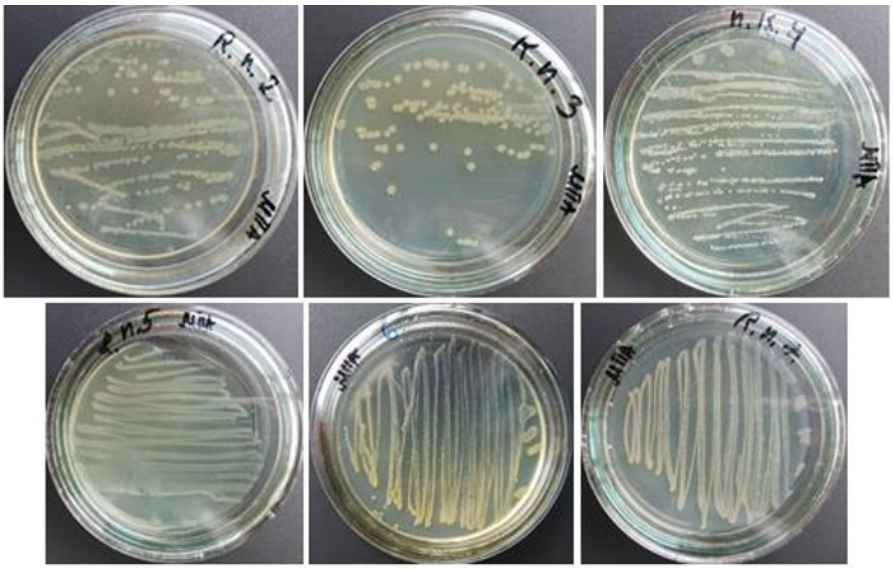

Figure 1. Qibray colonies of bacteria isolated from the stem and leaves of tomatoes brought from the greenhouse.

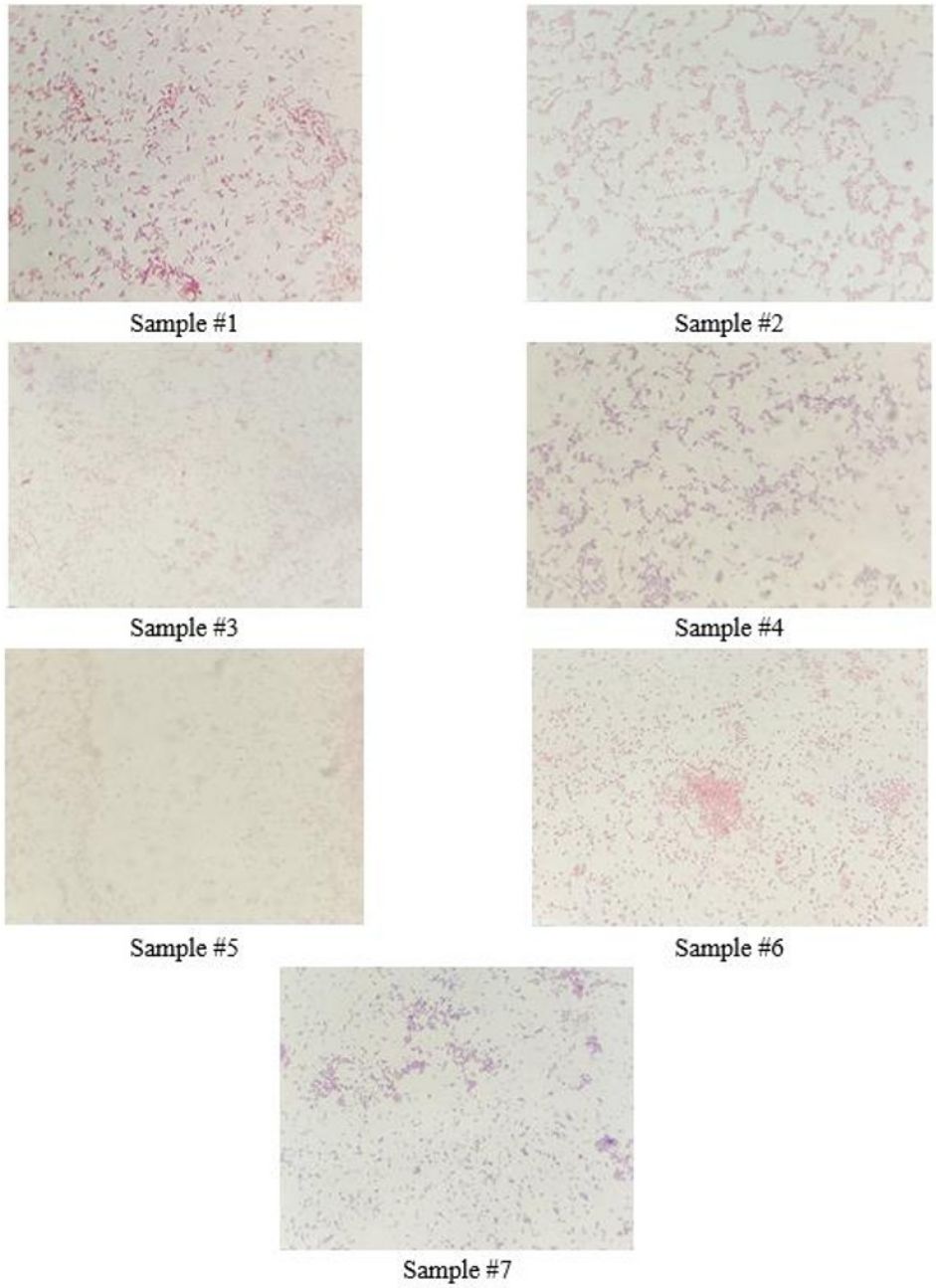

Figure 2. Qibray, TashGRES microscopic image of bacteria isolated from the stem and leaves of tomatoes brought from the greenhouse (magnified 1000 times).

Table 2. Qibray, TashGRES morphology of bacteria isolated from the stem and leaves of tomatoes brought from the greenhouse.

Isolates	Morphology
Pseudomonas antarctica 2/9	Gramophone, tieshaped, 2-4 microns long, 0.8-1.0 microns wide.
Stenotrophomonas sp 2/13	Micrococcus, monococcus, tetracoccus, diplococcus. Sarsina. In the form of streptococcus. Gram-negative, diameter 1.1 microns.
Pseudomonas sp. 2/11	Gram-negative, small rod-shaped bacteria, height 1.8-3.0 microns, width 0.6-0.8 microns
Paenarhrobacter ilicis 2/12	Gram-positive, Tae-shaped, 2-4 microns long and 1.0 microns wide.
Stenotrophomonas sp 2/10	Gram-negative, very small stick or Coca-Cola. Height 1.2 microns, width 0.8-1.0 microns.
Sphingobacterium faecium 2/14	Gram-negative small scales, no more than 1.5 microns in size, they are 1.2 microns.
Microbacterium phyllosphaerae 2/15	Gram-positive, some slightly curved, height 1.5-2.0 microns, width 1.0 microns.

The morphology of other isolated isolates was studied in a similar manner. To determine the pathogenic properties of the bacteria, they were grown in liquid feed for 15 days. With a mass formed by bacteria, vegetable seeds were subjected to intravenous spraying for 1 day. The finished seeds were placed in wet chambers. 10 tomato seeds were planted in wet chambers in each Petri dish. The experiments were monitored daily. According to the results of the study, the following table was formed (Table 3).

Table 3. "Yusuf" pathogenic properties of bacteria isolated from the affected parts of varietal tomatoes during the growing season.

#	Strain	Total planted tomato seeds	From these germinated seeds of the don	Not germinated seed	Stem length, relative to control, average	Root length relative to control, medial mm
1	Control	10	10	0	55	90.5
2	Microbacterium paraoxydans 3/14	10	7	3	33.5	56
3	*Stenotrophomonas maltophilia* 2/19	10	2	8	1.1	22.5
4	*Stenotrophomonas* sp. 2/13	10	5	5	2.5	36.5
5	*Klibsiella oxytoca* 6/26	10	8	2	30.5	45.5
6	*Sphingobacterium faecium* 2/14	10	8	2	31.5	75
7	*Bacteroides ovatus* 4/22	10	3	7	20	24.5
8	*Pseudomonas antarctica* 2/9	10	8	2	31.5	63
9	*Brevundimonas diminuta* 2/20	10	4	6	13.5	21.5
10	*Pseudomonas putida* 4/23	10	3	7	13.5	19.5
11	*Microbacterium phyllosphaerae* 2/15	10	8	2	38.5	57.5

12	*Pseudomonas mendocina* 4/27	10	6	4	19	43
13	*Paenarhrobacter ilicis* 2/12	10	6	4	22.5	42.5
14	*Pseudomonas alcaliphila* 2/18	10	5	5	26	42.5
15	*Stenotrophomonas maltophilia* 2/16	10	5	5	7.5	19.5

In Table 4, to determine the pathogenicity of bacteria isolated from tomatoes, tomato seeds were planted in Petri dishes with artificial damage. 10 seed grains were planted in wet chambers in each Petri dish, infecting them with bacteria. Research data showed that 2.19, 4.22, 2.20, 4.23 negatively affected germination and seed growth when sown with bacterial strains. Strains 6.26, 2.14, 2.9, 2.15 did not have a negative effect on germination and seed growth. Thus, it turned out that not all bacteria isolated from vegetable crops are pathogenic.

Table 4. Pathogenic properties of bacteria isolated from eggplant parts infected during the growing season.

#	Strain	Total planted tomato seeds	Germinated seed	Not germinated seed	Rod length relative to the regulator, mm	Length of the root relative to the control, mm
1	Control	10	10	0	8	11
2	Microbacterium paraoxydans 3/14	10	10	0	8,6	17,5
3	*Stenotrophomonas maltophilia* 2/19	10	0	10	-	-
4	*Stenotrophomonas* sp. 2/13	10	1	9	-	0,3
5	*Klibsiella oxytoca* 6/26	10	7	3	4,8	11,8
6	*Sphingobacterium faecium* 2/14	10	4	6	2	4,3
7	*Bacteroides ovatus* 4/22	10	5	5	1,5	4,3
8	*Pseudomonas antarctica* 2/9	10	7	3	5,8	11,5
9	*Brevundimonas diminuta* 2/20	10	0	10	-	-
10	*Pseudomonas putida* 4/23	10	4	6	-	2,5
11	*Microbacterium phyllosphaerae* 2/15	10	7	3	1,3	6,2
12	*Pseudomonas mendocina* 4/27	10	5	5	1,6	8
13	*Paenarhrobacter ilicis* 2/12	10	0	10	-	-
14	*Pseudomonas alcaliphila* 2/18	10	6	4	1,2	5,3
15	*Stenotrophomonas maltophilia* 2/16	10	0	10	-	-

It was found that during artificial sowing of eggplant seeds by bacteria, strains 2/20, 2/16, 2/13, 2/12, 2/19 have a negative effect on fertility. It was found that 10 seed pods planted with 2/20, 2/16 strains of bacteria lost their tensile strength. This means that these fungi cause rotting of the roots of the plant during the germination period, when the plant is sown, during the growing season of the plant. When sowing infected with bacterial strains 6.26, 2.9, 2.15 isolated from vegetable crops, these bacteria did not have a negative effect on germination and seed growth.

4 Conclusions

In drawing our research to a close, it becomes evident that not all bacteria isolated from vegetable crops exhibited pathogenic tendencies towards agricultural plants. Conversely, specific strains, including 6.26, 2.14, 2.9, and 2.15, displayed a positive impact on the germination and growth of tomato and eggplant seeds during our experimentation. This intriguing revelation underscores the potential benefits of harnessing bacteria from vegetable crops for enhancing seed germination and overall plant development.

Furthermore, the isolation and identification of these bacterial strains lay the foundation for the potential development of novel biological preparations. By isolating bacterial species with robust antimicrobial properties, which surpass their pathogenic counterparts, we open avenues for the formulation of biologically derived substances. These substances could potentially serve as alternatives to traditional chemical interventions in agriculture, fostering a more sustainable and environmentally friendly approach to crop protection.

In essence, our findings underscore the multifaceted nature of bacteria isolated from vegetable crops, highlighting both their positive potential and their non-pathogenic attributes. As we navigate towards agricultural practices that prioritize both productivity and ecological harmony, the utilization of these bacterial strains may offer innovative solutions to crop management and plant health enhancement.

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