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ORIGINAL ARTICLE

Biodiversity and scope of endophytic and phytopathogenic bacterial species identified in plant samples investigated in the Plant Disease Clinic laboratory

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Abstract

Modern agriculture and plant breeding must continuously meet the high and increasingly growing requirements of consumers and recipients. In this context, one of the conditions for effective management of any farm is access to quick and efficient diagnostics of plant pathogens, the result of which, together with the assessment of experts, provide breeders with tools to effectively reduce the occurrence of plant diseases. This paper presents information about biodiversity and spectrum of endophytic and phytopathogenic bacterial species identified in plant samples delivered to the Plant Disease Clinic in 2013–2019. During the tests, using the Biolog Gen III system, the species affiliation of the majority of detected bacterial strains found in plant tissues as an endophyte and not causing disease symptoms on plants was determined. These data were compiled and compared with the number of found identifications for a given species and data on the pathogenicity of bacterial species towards plants. In this way, valuable information for the scientific community was obtained about the species composition of the bacterial microbiome of the crop plants studied by us, which were confronted with available literature data. In the study, special attention was paid to tomato, which is the plant most often supplied for testing in the Plant Disease Clinic due to its economic importance.

Keywords: bacterial plant diseases, clinic, diagnostics, pathogen, tomato

Introduction

Bacterial pathogens cause significant losses in Polish agriculture (Pulawska *et al.* 2000; Pospieszny *et al.* 2007; Krawczyk *et al.* 2010, 2016; Mikiciński *et al.* 2010, 2016; Zwolińska *et al.* 2011, 2012, 2016; Iakimova *et al.* 2013; Kalużna *et al.* 2014, 2016; Krawczyk and Borodynko-Filas 2020; Krawczyk and Łochyńska 2020) and around the world (Freeman and Pataky 2001; Esker and Nutter 2002; Coutinho and Venter 2009; Rapicavoli *et al.* 2018; Xin *et al.* 2018; Nabhan *et al.* 2019). Despite this fact, modern agriculture and plant breeding must meet the high and increasingly growing requirements of consumers and recipients. These requirements are dictated by strict phytosanitary regulations in force concerning the marketing of plant products and by the expectations and awareness of consumers – the final recipients of plant-growing products. With long term usage of chemical crop protection, the products seem to reach the limits of their efficacy, beyond which no increase in yields is observed. Excessive usage of chemical crop protection products leads to adverse and often almost irreversible changes in the agricultural ecosystem. The introduction of integrated plant protection required by European Union regulations (from 2014) is expected to remedy these changes. These regulations aim to maximize the potential of biological plant protection while reducing the amount of chemical plant

protection products used. Increasing the biodiversity of agricultural agrosystems may decrease the number of observed plants showing disease symptoms caused by various pests and plant pathogens. This process has become particularly important in recent years. Climate change has resulted in the emergence of new diseases in Polish crops that previously had not posed a threat. In this context, effective management of any professional farm requires access to quick and efficient diagnostics of plant pathogens, which, together with the assessment of experts, can provide breeders with tools to effectively reduce the occurrence of plant diseases. In response to the demands of agriculture and society, the Plant Protection Clinic was established at the Institute of Plant Protection, in Poznań. The Clinic is the only accredited institution in Poland providing services in the field of identification of pathogens causing viral, bacterial and fungal diseases on agricultural, vegetable and ornamental plants, based on biological and molecular diagnostic methods. The laboratory was co-funded by European Union resources in March 2011, as part of the project "Modernization of laboratories to strengthen the innovation of research in the field of plant protection and economic activities" (WND-POIG.02.01.00-30--069/09-01). The laboratory is located in Poznań. Together with the Bank of Plant Pathogens it forms a separate department – "Plant Disease Clinic and Bank of Plant Pathogens" of the Institute of Plant Protection – National Research Institute. The role of the Clinic is to meet expectations of growers and use the experience and scientific resources of the institute to provide clients with high quality services of professional diagnostics of virological, bacteriological and mycological diseases of crop plants. As a result of the efforts and continuous improvement of the entire staff, the Clinic obtained accreditation by the Polish Centre of Accreditation (PCA), in the scope of our research methods. The Clinic obtained the credentials of an accredited laboratory on May 16, 2013 and currently uses accreditation number AB 1435.

The purpose of this article is to provide the reader with information about the biodiversity and scope of endophytic and phytopathogenic bacterial species identified in samples submitted to the Plant Disease Clinic (PDC) in the years 2013–2019. This study was aimed to provide the scientific community with insight into the scope of bacterial species identified in the tissues of various plant samples investigated in the Clinic.

According to the data of the Polish Central Statistical Office (SP 2018), Poland is the sixth-largest tomato producer in the European Union and nearly 70% of all cultivation is located in Greater Poland, Kuyavian-Pomeranian, Mazovian and Lublin voivodeships. As a result, the tomato (*Solanum lycopersicon*) is the most frequently tested crop in PDC.

Materials and Methods

In the PDC, the plant samples were registered, including the approximate location of their origin. The samples were then examined for the presence of bacteria, viruses and fungi pathogenic to plants. The technique of working with bacteria was as follows; plant fragments with disease symptoms were rinsed with distilled water to wash away residual of natural impurities. Then, the washed tissues were disinfected by immersion, for 1 min, in the following solutions: 10% hydrogen peroxide (H_2O_2), 70% ethyl alcohol (C_2H_5OH), and 5% sodium hypochlorite (NaOCl). Next, the disinfected plant parts were rinsed three times in sterile distilled water (SDW) to remove any disinfectant residues. After rinsing, pieces of the plant tissues at the junction of the healthy and diseased areas were dissected with a sterile scalpel and homogenized in sterile bags for homogenization of samples (Bioreba) in 3 ml of SDW. A series of decimal dilutions were made in SDW, from the obtained homogenizates, and aliquots of 0.1 ml of 10^{-1}, 10^{-2} and 10^{-3} dilutions were streaked onto Petri dishes containing Tryptic Soy Agar (TSA) (Sigma Aldrich Ltd.). The bacterial suspension was spread on the medium with a glass rod and incubated for 48 h at 27°C. The efficacy of the sterilization process was verified by plating 0.1 ml of sterile distilled water from the last rinsate on the TSA medium. The lack of colonies grown after incubation indicated effective sterilization.

From the dissected plant tissues the bacterial colonies were grown on TSA medium. For each sample, three quantitatively dominant morphotypes were isolated, and by using a series of reduction streaks, from each morphotype a pure culture strain was obtained. The cultures were Gram-stained and their purity was verified under an optical microscope. Next, the strains were identified using a Biolog Gen III system (BIOLOG Inc. Hayward, CA) according to the manufacturer's instructions. The Biolog Gen III system is a ready-made diagnostic kit containing powdered media on a 96-well plate to identify Gram-positive and Gram-negative bacteria. The Biolog Gen III test panel contained 71 carbon sources and 23 chemicals to determine the sensitivity of the isolate to be tested. The Gen III system analyzed the ability of a bacterial cell to metabolize all major classes of compounds and enabled the determination of other important physiological properties, such as functioning at a certain pH, salinity and lactic acid tolerance, reducing power and

chemical sensitivity of the examined bacterial strain. As a result of the analysis, a unique biochemical profile of 95 characteristics of the examined strain was obtained for each bacterial strain. The profile was verified against the Biolog Gen III system`s database containing nearly 3,000 other biochemical profiles, including those of phytopathogenic bacteria. The algorithm, based on the differences between individual biochemical profiles, estimated the degree of kinship of the tested isolates and expressed it in special units. On this basis, the bacterium under study was identified and the names of species with the closest biochemical profile were also given (Chojniak *et al.* 2015). Tested bacterial strains were inoculated onto BUG medium (BIOLOG Inc. Hayward, CA) and incubated for 24 h at 27°C. Bacterial colonies which grew after incubation were suspended in inoculation fluid (IF-A) (BIOLOG Inc. Hayward, CA) to prepare a suspension at the manufacturer's specified bacterial concentration. An aliquot of 0.1 ml of this suspension was then applied to each well in a 96 well of Gen III plate and incubated at the temperature optimal for the tested strain, which is 27°C for most bacterial plant pathogens (Schaad *et al.* 2001). After incubation, a microplate was placed in a dedicated reader where, based on spectrophotometric measurements, the system measured the optical density (OD_{595} nm) in each well. This biochemical profile was verified against the Biolog Gen III system database. The identification result was given in the form of the name of the bacterial species with a biochemical profile contained in the Biolog database that was closest to the profile of the isolate under study. The database v. 2.8.0. that we used contained biochemical profiles of 1568 species of aerobic bacteria, including profiles of most known bacterial plant pathogens. When necessary, a pathogenicity test was performed to confirm the pathogenic properties of the test strain. The appropriate kind of test and a host plant were selected for each sample individually. In PDC, the most commonly used test is a hypersensitivity reaction on tobacco plants, because many phytopathogenic bacteria can cause this effect. The test was performed as follows: from a 24-h (27°C) strain`s pure culture grown on BUG medium (Biolog Hayward, USA), a bacterial suspension with a concentration of 10^7 cfu · ml^{-1} was prepared in SDW. Next, tobacco plants were infected by injecting the suspension into the bottom side of the leaf blade, under the epidermis. The positive control was the reference strain of the bacterial plant-pathogen with known properties, e.g. *Pectobacterium carotovorum*. Negative controls were plants injected with sterile distilled water instead of the bacterial suspension. A positive result of the hypersensitivity reaction was manifested in the form of necrosis, observed 48–72 h after inoculation.

Results

From the founding of the PDC until December of 2019, a total of 394 samples were tested for the presence of plant-pathogenic bacteria. From the supplied plant samples, seven functional groups of plants were distinguished based on the manner of plant use. The groups were vegetables, fruits, ornamental plants, herbs, trees, shrubs and other plants that could not be explicitly qualified into one of the groups mentioned above.

In the vegetable group, the tomato was the most frequently studied plant (185 samples = 48%). Next were: cucumber (18 samples), onion (8), cauliflower (6), broccoli and carrots (4), beetroot (8), pepper (2), lettuce (2), white cabbage (2), asparagus (2), turnip (1), radish (1), kale (1) and Chinese cabbage (1).

Among the ornamental plants, geranium (8 samples) was the most numerous, followed by chrysanthemum (5), lily (5), begonia (4), primula (3), eustoma (3), violet (3), anthurium (2), gerbera (2), aster (2), phlox (1), anchusa (1), rose (1), cyclamen (1), brunnera (1), epimedium (1), navelwort (1), periwinkle (1), knotweed (1), anemone (1), clove (1), saxifrage (1), magnolia (1), surfinia (1), hellebore (1), bedbug (1), catnip (1), tiarella (1), craving (1) and anafalis (1).

The most frequently studied fruits were strawberry (6 samples) and blueberry (6) as well as raspberry (5), watermelon (3), apple tree (1) and Kamchatka berry (1).

Among the herbs, the largest number of samples was recorded for St. John's wort (3), sage (2), lavender (1) and basil (1).

The following trees and shrubs were examined: fruit tree rootstocks (3 samples), spruce (2), birch (1), willow (1), catalpa (1), boxwood (1), thuja (1) and juniper (1).

The most frequently examined agricultural plants included: sugar beet (6 samples), potato (5), pea (1), rape (1), soybean (1), corn (1) and barley (1).

In the last group of plants, not eligible for any of the above groups, the following were tested: echinodorus (aquarium plant) (2 samples), sedge (1), reed (1), peach (1), ivy (1), tobacco (1) and fungi (*Agaricus bisporus*) (1). The plant species most frequently studied in the PDC are listed in Table 1.

Since tomato was the most frequently tested plant in PDC, we investigated the percentage of the tested samples (a total of 185) per voivodeship. The region with the largest percent was Greater Poland (45% of tested tomato samples), next Lower Silesia (27%) and Masovian voivodeship (Fig. 1).

During the tests, using the Biolog Gen III system, not only was the presence or absence of a bacterial pathogen in the sample determined, but also the species identity of each tested strain was assessed, if there

Table 1. Plant species most frequently examined in the Plant Disease Clinic

Species of the tested plant	Quantity of tested samples
Tomato (*Solanum lycopersicum* L.)	185
Cucumber (*Cucumis sativus* L.)	18
Beetroot (*Beta vulgaris* L.)	8
Onion (*Allium cepa* L.)	8
Blueberry (*Vaccinium* L.)	6
Cauliflower (*Brassica oleracea* L. var. *botrytis* L.)	6
Pelargonia (*Pelargonium* L'Hér.)	6
Strawberry (*Fragaria ananassa* Duchesne)	6
Chrysanthemum (*Dendranthema* Des Moul)	5
Lily (*Lilium* L.)	5
Raspberry (*Rubus* L.)	5
Potato (*Solanum tuberosum* L.)	5
Carrot (*Daucus carota* L.)	4
Broccoli (*Brassica oleracea* L. var. *italica* Plenck)	4

Fig. 1. The percentage (%) of tested tomato samples per voivodeship

was a matching reference in the Biolog`s database. In this way, all the pathogenic bacterial strains and most of the accompanying endophytic strains found in plant tissues were identified biochemically. These data were summarized in Tables 2–7 and aligned with the data on the number of identified bacterial species, the number of identification cases and with information as to whether a given strain is a plant pathogen or not.

Both detecting the presence of fungi and viruses in plant samples, and the presence of phytopathogenic bacteria are basic objectives of the Clinic's activity. The data collected in the years 2013–2019 made it possible

Table 2. Bacterial species isolated from vegetable plants and identified using the Biolog Gen III system (v. 2.8.0)

No.	Tested plant	Identified bacterial species	Quantity of identifications	Plant pathogen (yes/no)
1.	Tomato (*Solanum lycopersicum*)	*Microbacterium maritypicum*	22	no
		Stenotrophomonas rhizophila	21	no
		Pseudomonas fluorescens	20	no
		Pseudomonas putida	16	no
		Pantoea dispersa	14	no
		Clavibacter michiganensis	11	yes
		Pseudomonas syringae pv. *syringae*	9	yes
		Pseudomonas viridilivida	9	yes
		Bacillus pseudomycoides / cereus	8	no
		Pectobacterium carotovorum subsp. *carotovorum*	8	yes
		Serratia fonticola	8	no
		Enterobacter cloacae subsp. *dissolvens*	7	no
		Pantoea agglomerans	7	yes
		Rhizobium radiobacter	7	yes
		Pseudomonas aeruginosa	6	no
		Ralstonia pickettii	6	no
		Serratia liquefaciens / grimesii	6	no
		Stenotrophomonas maltophilia	6	no
		Bacillus pumilus / safensis	6	no
		Klebsiella oxytoca	5	no
		Pantoea ananatis	5	yes
		Pseudomonas fluorescens	5	no

Table 2. Bacterial species isolated from vegetable plants and identified using the Biolog Gen III system (v. 2.8.0) – continuation

No.	Tested plant	Identified bacterial species	Quantity of identifications	Plant pathogen (yes/no)
	Tomato (*Solanum lycopersicum*)	*Pseudomonas plecoglossicida*	5	no
		Pseudomonas tolaasii	5	yes
		Bacillus marisflavi	4	no
		Brachybacterium alimentarium	4	no
		Chryseobacterium culicis	4	no
		Empedobacter brevis	4	no
		Escherichia vulneris	4	no
		Pseudomonas marginalis	4	yes
		Acinetobacter guillouiae	3	no
		Brachybacterium phenoliresistens	3	no
		Burkholderia pyrrocinia / cepacia	3	no
		Curtobacterium flaccumfaciens	3	yes
		Enterobacter cancerogenus	3	no
		Microbacterium saperdae	3	no
		Pantoea eucrina	3	no
		Providencia alcalifaciens	3	no
		Pseudomonas alcaligenes	3	no
		Pseudomonas cichorii	3	yes
		Pseudomonas corrugata	3	yes
		Serratia rubidaea	3	no
		Sphingobacterium multivorum	3	no
		Brevibacterium epidermidis	3	no
		Enterobacter cloacae	3	no
		Acinetobacter baumannii / pittii	2	no
		Acinetobacter gyllenbergii	2	no
		Aeromonas caviae	2	no
		Arthrobacter globiformis	2	no
		Bacillus oleronius	2	no
		Bacillus ruris	2	no
		Brachybacterium rhamnosum	2	no
		Brevibacterium casei	2	no
		Clavibacter michiganensis subsp. *michiganensis*	2	yes
		Enterobacter aerogenes (Klebsiella mobilis)	2	no
		Enterobacter nimipressuralis	2	no
		Exiguobacterium undae	2	no
		Klebsiella variicola	2	no
		Kluyvera intermedia	2	no
		Paenibacillus xylanilyticus	2	no
		Raoultella planticola / ornithinolytica	2	no
		Sphingomonas paucimobilis	2	no
		Staphylococcus epidermidis	2	no
		Staphylococcus pasteuri	2	no
		Staphylococcus sciuri subsp. *sciuri*	2	no
		Staphylococcus warneri	2	no
		Tetragenococcus halophilus subsp. *halophilus*	2	no
		Xanthomonas axonopodis	2	yes
		Bacillus atrophaeus / subtilis	2	no
		Acinetobacter beijerinckii	1	no
		Acinetobacter genomospecies	1	no
		Acinetobacter johnsonii	1	no
		Aeromonas hydrophila subsp. *anaerogenes*	1	no
		Aeromonas hydrophila subsp. *hydrophila*	1	no
		Arthrobacter histidinolovorans	1	no
		Arthrobacter woluwensis	1	no
		Bacillus amyloliquefaciens subsp. *amyloliquefaciens*	1	no
		Bacillus indicus	1	no
		Bacillus nealsonii	1	no

Table 2. Bacterial species isolated from vegetable plants and identified using the Biolog Gen III system (v. 2.8.0) – continuation

No.	Tested plant	Identified bacterial species	Quantity of identifications	Plant pathogen (yes/no)
	Tomato	*Bacillus safensis*	1	no
	(*Solanum lycopersicum*)	*Bordetella trematum*	1	no
		Brevibacterium sanguinis	1	no
		Burkholderia ambifaria / cepacia	1	no
		Cedecea neteri	1	no
		Chryseobacterium balustinum	1	no
		Chryseobacterium scophthalmum	1	no
		Chryseobacterium taichungense	1	no
		Citrobacter freundii	1	no
		Citrobacter sedlakii	1	no
		Citrobacter werkmanii	1	no
		Clavibacter michiganensis subsp. *tessellarius*	1	yes
		Comamonas testosteroni	1	no
		Corynebacterium kroppenstedtii	1	no
		Cupriavidus gilardii	1	no
		Cupriavidus pauculus	1	no
		Curtobacterium flaccumfaciens pv. *oortii*	1	yes
		Curtobacterium luteum	1	no
		Dermacoccus nishinomiyaensis	1	no
		Enterobacter amnigenus	1	no
		Erwinia rhapontici	1	yes
		Flavobacterium johnsoniae	1	no
		Janibacter hoylei / anophelis	1	no
		Jonesia denitrificans	1	no
		Kluyvera ascorbata	1	no
		Kocuria varians	1	no
		Leclercia adecarboxylata	1	no
		Macrococcus brunensis	1	no
		Microbacterium arborescens	1	no
		Microbacterium flavescens	1	no
		Microbacterium testaceum	1	no
		Mirobacterium maritypicum	1	no
		Moraxella lincolnii	1	no
		Novosphingobium capsulatum	1	no
		Ochrobactrum anthropi	1	no
		Ochrobactrum grignonense	1	no
		Paenibacillus alkaliterrae	1	no
		Paenibacillus anaericanus	1	no
		Paenibacillus provencensis	1	no
		Paenibacillus soli	1	no
		Paenibacillus stellifer	1	no
		Paenibacillus taichungensis / pabuli	1	no
		Paenibacullus popiliae	1	no
		Pantoea ananatis pv. *ananatis*	1	yes
		Pantoea cypripedii	1	yes
		Paraburkholderia caryophylli	1	no
		Pectobacterium atrosepticum	1	yes
		Pediococcus acidilactici	1	no
		Providencia stuartii	1	no
		Pseudomonas asplenii	1	yes
		Pseudomonas caricapapayae	1	yes
		Pseudomonas flavescens	1	yes
		Pseudomonas fragi	1	no
		Pseudomonas fulva	1	no
		Pseudomonas nitroreducens	1	no
		Pseudomonas oleovorans subsp. *oleovorans*	1	no

Table 2. Bacterial species isolated from vegetable plants and identified using the Biolog Gen III system (v. 2.8.0) – continuation

No.	Tested plant	Identified bacterial species	Quantity of identifications	Plant pathogen (yes/no)
	Tomato (*Solanum lycopersicum*)	*Pseudomonas stutzeri*	1	no
		Pseudomonas synxantha	1	no
		Pseudomonas syringae	1	yes
		Pseudomonas syringae pv. *daphniphylli*	1	yes
		Pseudomonas syringae pv. *helianthi*	1	yes
		Pseudomonas syringae pv. *maculicola*	1	yes
		Rathayibacter rathayi	1	no
		Rothia amarae	1	no
		Staphylococcus capitis subsp. *urealyticus*	1	no
		Staphylococcus cohnii subsp. *cohnii*	1	no
		Staphylococcus intermedius	1	no
		Staphylococcus sciuri subsp. *carnaticus*	1	no
		Staphylococcus xylosus	1	no
		Streptococcus oralis	1	no
		Tatumella punctata	1	no
		Xanthomonas hortorum pv. *pelargonii*	1	yes
		Yersinia intermedia	1	no
2.	Cucumber (*Cucumis sativus*)	*Enterobacter cloacae* subsp. *dissolvens*	6	no
		Pseudomonas plecoglossicida	3	no
		Pseudomonas putida	3	no
		Pseudomonas fluorescens	3	no
		Stenotrophomonas rhizophila	2	no
		Acidovorax avenae subsp. *avenae*	1	yes
		Acinetobacter baumannii / pittii	1	no
		Arthrobacter globiformis	1	no
		Enterobacter cloacae	1	no
		Exiguobacterium acetylicum	1	no
		Globicatella sanguinis	1	no
		Kosakonia cowanii	1	no
		Microbacterium maritypicum	1	no
		Microbacterium testaceum	1	no
		Pectobacterium carotovorum	1	yes
		Pseudomonas oryzihabitans	1	no
		Pseudomonas synxantha	1	no
		Rhizobium radiobacter	1	yes
		Staphylococcus epidermidis	1	no
3.	Onion (*Alium cepa*)	*Rahnella aquatilis*	3	no
		Burkholderia ambifaria / cepacia	2	no
		Burkholderia gladioli pv. *gladioli*	2	yes
		Klebsiella oxytoca	2	no
		Proteus penneri	2	no
		Klebsiella pneumoniae subsp. *pneumoniae*	1	no
		Paenibacillus lautus	1	no
		Pantoea dispersa	1	no
		Staphylococcus xylosus	1	no
4.	Caulifower (*Brassica oleracea* var. *botrytis*)	*Proteus mirabilis*	7	no
		Klebsiella oxytoca	2	no
		Acinetobacter soli	1	no
		Pseudomonas fluorescens	1	no
		Pseudomonas fulva	1	no
		Pseudomonas plecoglossicida	1	no
		Pseudomonas putida	1	no
		Pseudomonas viridilivida	1	yes
		Pseudomonas caricapapayae	2	yes
		Pseudomonas fluorescens	2	no

Table 2. Bacterial species isolated from vegetable plants and identified using the Biolog Gen III system (v. 2.8.0) – continuation

No.	Tested plant	Identified bacterial species	Quantity of identifications	Plant pathogen (yes/no)
5.	Broccoli (*Brassica oleracea* var. *italica*)	*Cosenzaea myxofaciens*	1	no
		Enterococcus faecalis	1	no
		Ewingella americana	1	no
		Proteus hauseri	1	no
		Providencia heimbachae	1	no
		Pseudomonas fragi	1	no
		Pseudomonas plecoglossicida	1	no
6.	Carrot (*Daucus carota*)	*Serratia plymuthica*	3	no
		Pseudomonas putida	1	no
		Pantoea agglomerans	1	yes
		Pseudomonas tolaasii	1	yes
		Rahnella aquatilis	1	no
		Pseudomonas fluorescens	1	no
		Pseudomonas plecoglossicida	1	no
7.	Beetroot (*Beta vulgaris*)	*Pseudomonas putida*	3	no
		Achromobacter ruhlandii / denitrificans	1	no
		Enterobacter cloacae	1	no
		Kluyvera ascorbata	1	no
		Pseudomonas fluorescens	1	no
8.	Pepper (*Pipper nigrum*)	*Pectobacterium carotovorum*	1	yes
9.	Lettuce (*Lactuca* sp.)	*Enterobacter cloacae* subsp. *dissolvens*	1	no
		Pseudomonas putida	1	no
		Serratia ficaria	1	no
		Stenotrophomonas maltophilia	1	no
		Serratia plymuthica	3	no
		Pseudomonas fluorescens	2	no
10.	Asparagus (*Asparagus officinalis*)	*Achromobacter spanius*	1	no
		Acinetobacter nosocomialis	1	no
		Bacillus pumilus / safensis	1	no
		Bacillus subtilis	1	no
		Chryseobacterium balustinum	1	no
		Enterobacter amnigenus	1	no
		Enterobacter cloacae subsp. *dissolvens*	1	no
		Lysinibacillus boronitolerans	1	no
		Pseudomonas marginalis	1	yes
		Pseudomonas nitroreducens	1	no
		Pseudomonas tolaasii	1	yes
		Rahnella aquatilis	1	no
		Serratia liquefaciens / grimesii	1	no
		Stenotrophomonas rhizophila	1	no
11.	Turnip (*Brassica rapa* subsp. *rapa*)	*Enterobacter amnigenus*	1	no
		Pectobacterium carotovorum subsp. *carotovorum*	1	yes
12.	Radish (*Raphanus sativus*)	*Pseudomonas plecoglossicida*	1	no
		Raoultella planticola / ornithinolytica	1	no
		Serratia liquefaciens / grimesii	1	no
13.	White cabbage (*Brassica oleracea* var. *capitata*)	*Pseudomonas fulva*	1	no
		Pseudomonas marginalis	1	yes
		Xanthomonas hortorum pv. *carotae*	1	yes
		Xanthomonas arboricola pv. *juglandis*	1	yes
14.	Chinese cabbage (*Brassica rapa* subsp. *pekinensis*)	*Pseudomonas tolaasii*	1	yes
		Exiquobacterium acetylicum	1	no

Table 3. Bacterial species isolated from ornamental plants and identified with the Biolog Gen III system (v. 2.8.0)

No.	Tested plant	Identified bacterial species	Quantity of identification	Plant pathogen (yes/no)
1.	Geranium (*Pelargonium* sp.)	*Microbacterium maritypicum*	2	no
		Pantoea cypripedii	2	yes
		Bacillus pseudomycoides / cereus	1	no
		Brevibacterium otitidis	1	no
		Curtobacterium flaccumfaciens	1	yes
		Enterobacter hormaechei	1	no
		Klebsiella oxytoca	1	no
		Paenibacillus anaericanus	1	no
		Paenibacillus glycanilyticus	1	no
		Paenibacillus xylanilyticus	1	no
		Rhodococcus corynebacterioides	1	no
		Roseomonas cervicalis	1	no
		Xanthomonas hortorum pv. *pelargonii*	1	yes
2.	Chrysanthemum (*Chrysanthemum* sp.)	*Bacillus pumilus / safensis*	1	no
		Microbacterium testaceum	1	no
		Pantoea agglomerans	1	yes
		Pseudomonas fluorescens	1	no
		Pseudomonas viridilivida	1	yes
		Staphylococcus warneri	1	no
		Rahnella aquatilis	4	no
		Pseudomonas fluorescens	2	no
		Staphylococcus saprophyticus subsp. *bovis*	2	no
3.	Lily (*Lilium* sp.)	*Escherichia hermannii*	1	no
		Paenibacillus anaericanus	1	no
		Pantoea agglomerans	1	yes
		Pseudomonas fragi	1	no
		Pseudomonas syringae	1	yes
		Serratia liquefaciens / grimesii	1	no
		Stenotrophomonas rhizophila	1	no
4.	Primula (*Primula vulgaris*)	*Pseudomonas fluorescens*	2	no
		Pectobacterium carotovorum subsp. *carotovorum*	1	yes
		Pseudomonas cichorii	1	yes
		Raoultella planticola / ornithinolytica	1	no
5.	Eustoma (*Eustoma russellianum*)	*Pseudomonas fluorescens*	2	no
		Staphylococcus haemolyticus	2	no
		Pseudomonas tolaasii	1	yes
		Pseudomonas viridilivida	1	yes
		Serratia liquefaciens / grimesii	1	no
		Staphylococcus hominis subsp. *novoniosepticus*	1	no
6.	Violet (*Viola* sp.)	*Pantoea dispersa*	1	no
		Pseudomonas cichorii	1	yes
		Pseudomonas fluorescens	1	no
		Rhizobium radiobacter	1	yes
7.	Begonia (*Begonia obliqua*)	*Microbacterium testaceum*	3	no
		Aquaspirillum peragrinum subsp. *integrum*	2	no
		Paenibacillus polymyxa	2	no
		Bacillus agaradhaerens	1	no
		Bacillus cereus / pseudomyoides	1	no
		Bacillus licheniformis	1	no
		Curtobacterium luteum	1	no
		Kocuria rhizophila	1	no
		Microbacterium arborescens	1	no
		Microbacterium lacticum	1	no

Table 3. Bacterial species isolated from ornamental plants and identified with the Biolog Gen III system (v. 2.8.0) – continuation

No.	Tested plant	Identified bacterial species	Quantity of identification	Plant pathogen (yes/no)
	Begonia (*Begonia obliqua*)	*Microbacterium saperdae*	1	no
		Oerskovia turbata	1	no
		Paenibacillus sabinae	1	no
		Staphylococcus sciuri subsp. *sciuri*	1	no
8.	Anthurium (*Anthurium* sp.)	*Microbacterium imperiale*	1	no
		Paenibacillus provencensis	1	no
		Pseudomonas aeruginosa	1	no
		Rhizobium radiobacter	1	yes
9.	Geranium (*Pelargonium* sp.)	*Pantoea agglomerans*	1	yes
		Pantoea dispersa	1	no
		Paraburkholderia caribensis	1	no
10.	Gerbera (*Gerbera* sp.)	*Citrobacter freundii*	1	no
		Achromobacter ruhlandii / denitrificans	1	no
11.	Aster (*Aster* sp.)	*Acinetobacter soli*	1	no
		Enterobacter cloacae subsp. *dissolvens*	1	no
		Pseudomonas putida	1	no
		Staphylococcus capitis subsp. *capitis*	1	no
		Staphylococcus epidermidis	1	no
12.	Phlox (*Phlox* sp.)	*Acinetobacter haemolyticus*	1	no
		Acinetobacter soli	1	no
13.	Anchusa (*Anchusa azurea*)	*Pantoea agglomerans*	1	yes
		Pseudomonas caricapapayae	1	yes
		Pseudomonas plecoglossicida	1	no
14.	Rose (*Rosa* sp.)	*Bacillus vallismortis / subtilis*	1	no
15.	Cyclamen (*Cyclamen persicum*)	*Pantoea agglomerans*	1	yes
16.	Bruner (*Brunnera* sp.)	*Pantoea agglomerans*	1	yes
		Rhizobium radiobacter	1	yes
		Stenotrophomonas rhizophila	1	no
17.	Epimedium (*Epimedium grandiflorum*)	*Bacillus subtilis* subsp. *subtilis*	1	no
18.	Omphalodes (*Omphalodes verna*)	*Enterobacter cloacae* subsp. *dissolvens*	3	no
		Pseudomonas caricapapayea	1	no
		Pseudomonas viridilivida	1	yes
19.	Periwinkle (*Vinca* sp.)	*Enterobacter aerogenes (Klebsiella mobilis)*	1	no
		Pantoea agglomerans	1	yes
		Pseudomonas flavescens	1	yes
20.	Water pepper (*Persicaria hydropiper*)	*Pseudomonas tolaasii*	1	yes
21.	Anemone (*Anemone nemorosa*)	*Enterobacter cloacae*	1	no
		Exiguobacterium undae	1	no
		Pseudomonas tolaasii	1	yes
22.	Carnation (*Dianthus caryophyllus*)	*Acinetobacter soli*	1	no
		Paenibacillus anaericanus	1	no
		Pantoea dispersa	1	no
		Serratia ficaria	1	no
		Serratia liquefaciens / grimesii	1	no
23.	Saxifraga (*Saxifraga* sp.)	*Pantoea agglomerans*	1	yes

Table 3. Bacterial species isolated from ornamental plants and identified with the Biolog Gen III system (v. 2.8.0) – continuation

No.	Tested plant	Identified bacterial species	Quantity of identification	Plant pathogen (yes/no)
24.	Magnolia (*Magnolia grandiflora*)	*Staphylococcus warneri*	1	no
25.	Surfinia (*Petunia* sp.)	*Enterobacter cloacae* subsp. *dissolvens*	1	no
		Klebsiella oxytoca	1	no
		Pseudomonas marginalis	1	yes
		Pseudomonas viridilivida	1	yes
		Roseomonas cervicalis	1	no
26.	Hellebore (*Helleborus foetidus*)	*Pantoea dispersa*	1	no
		Pseudomonas fulva	1	no
		Stenotrophomonas rhizophila	1	no
27.	Black cohosh (*Actaea racemosa*)	*Acinetobacter nosocomialis*	1	no
		Acinetobacter soli	1	no
28.	Catnip (*Nepeta cataria*)	*Pantoea agglomerans*	1	yes
		Xanthomonas hortorum	1	yes
29.	Tiarella (*Tiarella polyphylla*)	*Enterobacter cloacae*	1	no
		Pseudomonas syringae	1	yes
		Xanthomonas euvesicatoria	1	yes
30.	Anaphalis (*Anaphalis margaritacea*)	*Escherichia vulneris*	1	no

to extract a list of 26 species of most commonly occurring plant pathogenic bacteria. These pathogens were detected in the following species of plants with a given frequency:

- *Clavibacter michiganensis* (14 detections on tomato),
- *Pseudomonas syringae* (17 detections on tomato, lily, tiarella and strawberry),
- *Pseudomonas viridilivida* (17 detections on tomato, cauliflower, chrysanthemum, eustoma, omphalodes, surfinia, strawberry and basil),
- *Pectobacterium carotovorum* (12 detections on tomato, cucumber, pepper, turnip and primula),
- *Pantoea agglomerans* (32 detections on tomato, chrysanthemum, lily, geranium, anchusa, cyclamen, brunnera, periwinkle, saxifraga, catnip, raspberry, apple, watermelon, blueberry, carrot, willow, catalpa, boxwood, thuja, juniper,potato, maize, carex, tobacco and fruit tree rootstocks),
- *Rhizobium radiobacter* (12 detections on tomato, cucumber, violet, anthurium, brunnera and carex),
- *Pantoea ananatis* (8 detections on tomato and raspberry),
- *Pseudomonas tolaasii* (15 detections on tomato, carrot, asparagus, Chinese cabbage, eustoma, water pepper, anemone, strawberry, soya and champignon),
- *Pseudomonas marginalis* (9 detections on tomato, asparagus, white cabbage, surfinia and strawberry),
- *Curtobacterium flaccumfaciens* (8 detections on tomato, geranium, blueberry, catalpa, white beet and ivy),
- *Pseudomonas cichorii* (7 detections on tomato, primula, violet and potato),
- *Pseudomonas corrugata* (3 detections on tomato),
- *Xanthomonas axonopodis* (2 detections on tomato),
- *Erwinia rhapontici* (4 detections on tomato, boxwood, thuja and pea),
- *Pantoea cypripedii* (3 detections on tomato and geranium),
- *Pectobacterium atrosepticum* (1 detection on tomato),
- *Pseudomonas asplenii* (1 detection on tomato),
- *Pseudomonas caricapapayae* (5 detections on tomato, cauliflower, anchusa and rape),
- *Pseudomonas flavescens* (4 detections on tomato, periwinkle and sedge),
- *Xanthomonas hortorum* (5 detections on tomato, white cabbage, geranium, catnip and sage),
- *Acidovorax avenae* (1 detection on cucumber),
- *Burkholderia gladioli* (1 detection on onion),
- *Xanthomonas arboricola* (1 detection on white cabbage),
- *Xanthomonas euvesicatoria* (1 detection on tiarella),
- *Xanthomonas oryzae* (1 detection on sedge),
- *Lelliottia amnigena* (7 detections on fruit tree rootstocks and white beet),
- *Kosakonia cowanii* (11 detections on soya and dracaena).

The most frequently identified plant pathogens and their percentage in total samples tested is presented in Figure 2.

Table 4. Bacterial species isolated from fruit plants and identified with the Biolog Gen III system (v. 2.8.0)

No.	Tested plant	Identified	Quantity of identification	Plant pathogen (yes/no)
1.	Strawberry (*Fragaria vesca*)	*Pseudomonas fluorescens*	4	no
		Pseudomonas viridilivida	2	yes
		Acinetobacter guillouiae	1	no
		Bacillus licheniformis	1	no
		Enterobacter amnigenus	1	no
		Pseudomonas marginalis	1	yes
		Pseudomonas plecoglossicida	1	no
		Pseudomonas synxantha	1	no
		Pseudomonas syringae	1	yes
		Pseudomonas syringae pv. *theae*	1	yes
		Pseudomonas tolaasii	1	yes
		Stenotrophomonas rhizophila	1	no
2.	Blueberry (*Vaccinium corymbosum*)	*Pantoea agglomerans*	2	yes
		Bacillus pumilus / safensis	1	no
		Curtobacterium flaccumfaciens	1	yes
		Citrobacter freundii	1	no
		Ewingella americana	1	no
3.	Raspberry (*Rubus idaeus*)	*Pantoea agglomerans*	3	yes
		Enterobacter nimipressuralis	2	no
		Stenotrophomonas rhizophila	2	no
		Pantoea ananatis	2	yes
		Serratia liquefaciens / grimesii	1	no
		Pantoea eucrina	1	no
		Micrococcus luteus	1	no
		Pseudomonas fulva	1	no
		Stenotrophomonas maltophilia	1	no
		Enterobacter amnigenus	1	no
		Bacillus gibsonii / murimartini	1	no
4.	Watermelon (*Citrullus lanatus*)	*Pseudomonas putida*	5	no
		Siccibacter turicensis	2	no
		Acinetobacter lwoffii	1	no
		Pantoea agglomerans	1	yes
		Enterobacter aerogenes (Klebsiella mobilis)	1	no
		Acinetobacter guillouiae	1	no
		Microbacterium maritypicum	1	no
5.	Apple (*Malus domestica*)	*Enterobacter amnigenus*	3	no
		Pantoea agglomerans	3	yes
6.	Kamchatka blueberry (*Lonicera caerulea*)	*Bacillus marisflavi*	1	no

Additionally, the percentage of bacterial pathogen detections for the most frequently studied plant species is presented below for tomato (Table 8) and other plants (Table 9).

Discussion

Professional plant breeders and producers make up the majority of the Clinic`s clients. The other clients are owners of individual horticultural and agricultural farms. Orders from individual private persons are a minority. Following this specific client`s profile, it is understandable that a relationship between tested plant species and the period of year is observed. For example, most chrysanthemums to be tested are commissioned in the autumn, before All Saints' Day. Ornamental plants are sent for examination mainly in the spring and summer, which coincides with their flowering period. Tomatoes are commissioned for testing throughout most of the year, but mainly in the summer. In the autumn-winter period, tests for detecting the occurrence of pathogenic bacteria for plants are usually

Table 5. Bacterial species isolated from herbs and identified with the Biolog Gen III system (v. 2.8.0)

No.	Tested plant	Identified bacterial species	Quantity of identification	Plant pathogen (yes/no)
1.	St. John's wort (*Hypericum perforatum*)	*Staphylococcus epidermidis*	2	no
		Staphylococcus capitis subsp. *capitis*	1	no
		Paenibacillus harenae	1	no
2.	Sage (*Salvia officinalis*)	*Staphylococcus hominis* subsp. *hominis*	2	no
		Xanthomonas oryzae pv. *oryzae*	1	yes
		Xanthomonas hortorum pv. *carotae*	1	yes
3.	Lavender (*Lavandula* sp.)	*Pseudomonas fragi*	1	no
		Burkholderia caryophylli	1	no
4.	Basil (*Ocimum basilicum*)	*Pseudomonas viridilivida*	1	yes
		Pseudomonas fluorescens	1	no
		Cupriavidus gilardii	1	no

Table 6. Bacterial species isolated from trees and shrubs and identified using the Biolog Gen III system (v. 2.8.0)

No.	Tested plant	Identified bacterial species	Quantity of identification	Plant pathogen (yes/no)
1.	Fruit tree rootstocks	*Lelliottia amnigena*	3	no
		Pantoea agglomerans	1	yes
		Enterobacter cloacae subsp. *dissolvens*	1	no
		Rahnella aquatilis	1	no
		Aeromonas bestiarum	1	no
2.	Spruce (*Picea* sp.)	*Pseudomonas fluorescens*	2	no
		Pseudomonas alcaligenes	1	no
		Pseudomonas congelans	1	no
		Curtobacterium pusillum	1	no
		Acinetobacter johnsonii	1	no
3.	Birch-tree (*Betula* sp.)	*Staphylococcus warneri*	1	no
4.	Willow (*Salix* sp.)	*Pantoea agglomerans*	1	yes
5.	Catalpa (*Catalpa bignonioides*)	*Curtobacterium flaccumfaciens*	1	yes
		Pantoea dispersa	1	no
		Pantoea agglomerans	1	yes
6.	Boxwood (*Buxus* sp.)	*Kosakonia cowanii*	2	no
		Pseudomonas fulva	1	no
		Pseudomonas fluorescens	1	no
		Erwinia rhapontici	1	yes
		Pantoea agglomerans	1	yes
7.	Thuja (*Thuja* sp.)	*Kosakonia cowanii*	2	no
		Pseudomonas fulva	1	no
		Pseudomonas fluorescens	1	no
		Erwinia rhapontici	1	yes
		Pantoea agglomerans	1	yes
8.	Juniper (*Juniperus* sp.)	*Pantoea agglomeransi*	1	yes
		Burkholderia glathe	1	no
9.	Dracaena (*Dracaena fragrans*)	*Kosakonia cowanii*	2	yes
		Enterobacter cloacae	1	no
		Enterobacter cloacae subsp. *dissolvens*	1	yes

Table 7. Bacterial species isolated from plants that cannot be assigned to any of the above categories, identified with the Biolog Gen III (v. 2.8.0) system – other plants

No.	Tested plant	Identified bacterial species	Quantity of identification	Plant pathogen (yes/no)
1.	Echinodorus (*Echinodorus* sp.; aquarious plant)	*Pseudomonas straminea*	1	no
		Pseudomonas putida	1	no
		Brachybacterium phenoliresistens	1	no
		Citrobacter farmeri	1	no
2.	Calamagrostis (*Calamagrostis* sp.)	*Serratia marcescens* subsp. *marcescens*	2	no
3.	Sedge (*Carex* sp.)	*Pseudomonas flavescens*	2	yes
		Rhizobium radiobacter	1	yes
		Pantoea agglomerans	1	yes
		Serratia ficaria	1	no
		Enterobacter cloacae subsp. *dissolvens*	1	no
		Enterobacter cloacae	1	no
		Enterobacter cloacae subsp. *cloacae*	1	no
4.	Tassel (*Capsella bursa-pastoris*)	*Stenotrophomonas maltophilia*	1	no
		Tatumella citrea	1	no
		Stenotrophomonas rhizophila	1	no
5.	Ivy (*Hedera* sp.)	*Curtobacterium flaccumfaciens*	1	yes
6.	Champignon (*Agaricus bisporus*)	*Pseudomonas tolaasii*	2	yes
7.	Tobacco (*Nicotiana tabacum*)	*Serratia rubidaea*	1	no
		Pantoea agglomerans	1	yes
		Enterobacter aerogenes (Klebsiella mobilis)	1	no

Table 8. Per cent of a plant pathogens identified on 185 tomato samples tested in Plant Disease Clinic

Pathogenic bacteria identified on tomato	Identification quantity on tomato	% of tomato samples
Clavibacter michiganensis	14	7.57
Pseudomonas syringae	13	7.03
Pseudomonas viridilivida	9	4.86
Pectobacterium carotovorum	8	4.32
Pantoea agglomerans	7	3.78
Rhizobium radiobacter	7	3.78
Pantoea ananatis	6	3.24
Pseudomonas tolaasii	5	2.70
Curtobacterium flaccumfaciens	4	2.16
Pseudomonas marginalis	4	2.16
Pseudomonas cichorii	3	1.62
Pseudomonas corrugata	3	1.62
Xanthomonas axonopodis	2	1.08
Erwinia rhapontici	1	0.54
Pantoea cypripedii	1	0.54
Pectobacterium atrosepticum	1	0.54
Pseudomonas asplenii	1	0.54
Pseudomonas caricapapayae	1	0.54
Pseudomonas flavescens	1	0.54
Xanthomonas hortorum	1	0.54

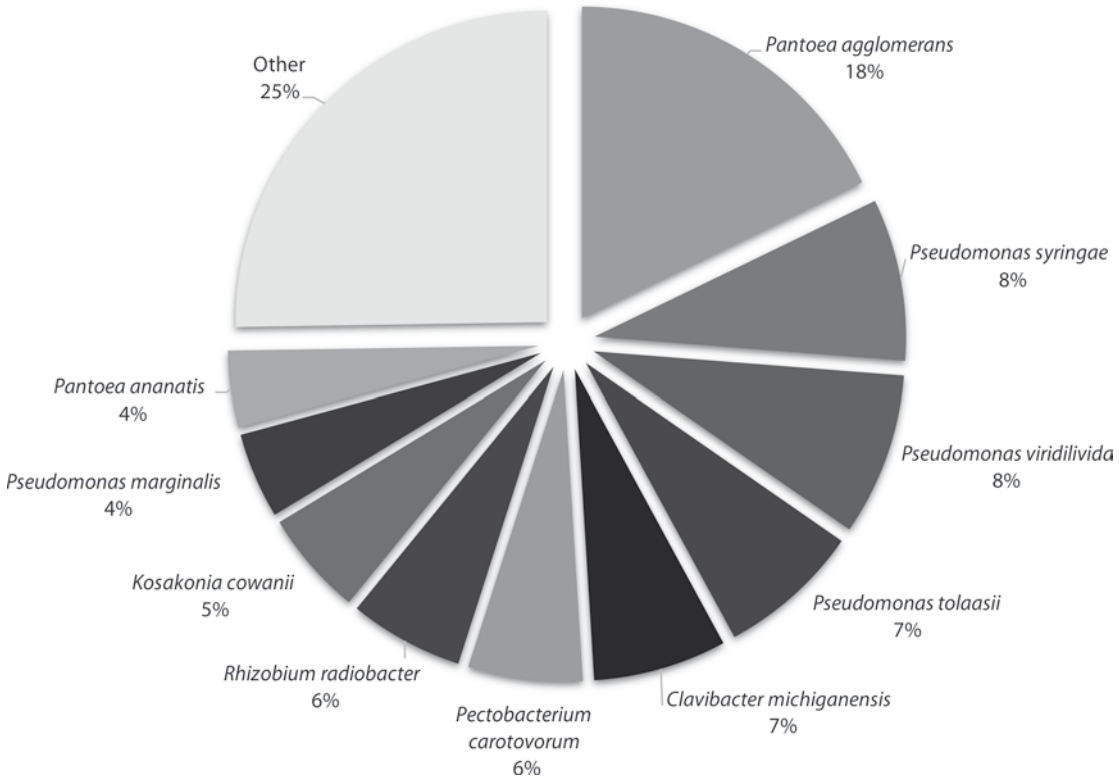

Fig. 2. The percentage of the 10 most frequently identified plant pathogens in a total number of 202 identifications of pathogenic bacterial strains isolated from 394 plant samples tested in the Plant Disease Clinic in 2013–2019

commissioned by producers growing their crops in greenhouses. The location distribution of the tested tomato samples presented in Figure 2 was affected by the proximity to the clinic. For this reason Greater Poland was most represented (45%). It also reflects the distribution of tomato growers in Poland, where in fact the three most frequently represented voivodeships are the main regions of tomato production (Fig. 2).

The scope of bacterial plant pathogens detected in the PDC (Fig. 1, Tables 2–3) is consistent with the list of the 10 most dangerous bacterial plant pathogens published by Mansfield *et al.* (2012). This list includes, starting with the most damaging crops: *Pseudomonas syringae* (all pathovars), *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris* (all pathovars), *Xanthomonas axonopodis* pv. *manihotis*, *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya dadantii* (and *solani*) and *Pectobacterium carotovorum* (and *P. atrosepticum*) (Mansfield *et al.* 2012). Among the 10 most dangerous bacterial plant pathogens, four of them were found in the clinic: *Pseudomonas syringae*, *Pectobacterium carotovorum*, *Xanthomonas axonopodis* and *Pectobacterium atrosepticum* (Table 2), which clearly shows that these pathogens are still a real threat for crops. In the tomato samples themselves, the presence of four of the listed pathogens was also noted (Table 2). Moreover, the presence of 16 other plant pathogen species was detected in the tomato samples under study, of which *Clavibacter michiganensis* was most frequently represented (Table 2). Also, the bacterial species causing the most common tomato diseases in Poland were observed, including *Pseudomonas syringae* pv. *tomato* (Buell *et al.* 2003; Zhao *et al.* 2003), bacterial leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Jones 1986; Ciardi *et al.* 2000), bacterial tomato cancer (*Clavibacter michiganensis* subsp. *michiganensis*) (Gartemann *et al.* 2003), pith necrosis (*Pseudomonas corrugata*) (Scarlett and Fletcher 1978; Lukezic 1979), bacterial wet rot (*Pectobacterium carotovorum*) (Daami-Remadi 2007; Ahmed *et al.* 2017) and crazy roots disease (*Rhizobium radiobacter*) (Sawada and Azegami 2014; Bosmans *et al.* 2017). The remaining bacterial species listed in Table 2 and identified in tomato samples are mentioned in the literature as pathogens of other crop plants and for champignon (*Agaricus bisporus*) in the case of *Pseudomonas tolaasii*. To our knowledge, previously, these bacteria had never been recorded as occurring on tomato. This is valuable information because it enriches our knowledge about the potential range of hosts of each bacterium and the range of pathogens for tomato. For the first time, we found the presence of the following bacterial species on tomato plants: *Pseudomonas viridilivida*, *Pantoea agglomerans*, *Pantoea ananatis*, *Pseudomonas tolaasii*, *Curtobacterium flaccumfaciens*, *Pseudomonas marginalis*, *Pseudomonas cichorii*, and

Table 9. The number of registered identifications of a plant pathogen per number of samples: cucumber, white beet, onion and blueberry

Plant host and pathogenic bacteria identified	Identification quantity	% of samples
Cucumber (18 samples)		
Acidovorax avenae	1	5.56
Pectobacterium carotovorum	1	5.56
Rhizobium radiobacter	1	5.56
Sugar beet (8 samples)		
Pseudomonas marginalis	2	25.00
Onion (8 samples)		
Burkholderia gladioli	2	25.00
Blueberry (6 samples)		
Curtobacterium flaccumfaciens	1	16.67
Pantoea agglomerans	2	33.33

Erwinia rhapontici. However, since these results were confirmed using only the Biolog Gen III system, this information needs to be confirmed by further testing using another method, e.g. a molecular method, like 16S rDNA sequencing.

In the case of cucumber, sugar beet, onion and blueberry, the observed profile of pathogenic bacteria was slightly different, because from the list of the 10 most dangerous bacterial pathogens only the presence of *Pectobacterium carotovorum* and *Rhizobium radiobacter* on cucumber was noted. Other pathogenic species identified were: *Acidovorax avenae*, *Pseudomonas marginalis*, *Burkholderia gladioli*, *Curtobacterium flaccumfaciens* and *Pantoea agglomerans*. These are known plant pathogens, however they occur much less frequently and with less economic importance.

During the research, in addition to data on the occurrence of pathogenic bacteria on plants, the occurrence of accompanying, cultivable, endophytic bacteria in particular plant species was also recorded. This information can be of great importance because it gives insight into the microbiome composition of multiple plant species. The identified bacteria are the most abundant strains grown on TSA medium after isolation from crude plant homogenate. Therefore, it needs to be assumed that those strains are the most likely to be a part of the microbiome of a tested plant. However, comparing a list of bacterial species included in tomato microbiome developed by other authors (Ottesen *et al.* 2013; Tian *et al.* 2017) with a list of bacterial species identified in tomato tissue samples examined in the Plant Disease Clinic in 2013–2019, significant differences can be observed. The number of cultivable bacterial species grown from the tomato tissues in the PDC laboratory is significantly higher than that found in the literature data (Table 10). A potential explanation for these differences may be the fact that a sample of tissue from the junction of healthy and diseased tissues is routinely taken for examination at the Clinic, which is dictated by the rules of proper conduct in

Table 10. Comparison of the species composition of healthy tomato microbiome with a list of bacterial species isolated from tomato plant tissues examined in the Plant Disease Clinic in 2013–2019

Healthy tomato microbiome according to literature	Bacterial species identified in tomato tissue samples tested in Plant Disease Clinic
(Tian *et al.* 2017)	*Microbacterium maritypicum*
Phylum Firmicutes:	*Stenotrophomonas rhizophila*
Bacillus amyloliquefaciens	*Pseudomonas fluorescens*
Bacillus anthracis	*Pseudomonas putida*
Bacillus bingmayongensis	*Pantoea dispersa*
Bacillus cereus	*Clavibacter michiganensis*
Bacillus methylotrophicus	*Pseudomonas syringae* pv. *syringae*
Bacillus pumilus	*Pseudomonas viridilivida*
Bacillus subtilis	*Bacillus pseudomycoides / cereus*
Staphylococcus epidermidis	*Pectobacterium carotovorum* subsp. *carotovorum*
Phylum Bulholderiales:	*Serratia fonticola*
Burkholderia cepacia	*Enterobacter cloacae* subsp. *dissolvens*

Table 10. Comparison of the species composition of healthy tomato microbiome with a list of bacterial species isolated from tomato plant tissues examined in the Plant Disease Clinic in 2013–2019 – continuation

Healthy tomato microbiome according to literature	Bacterial species identified in tomato tissue samples tested in Plant Disease Clinic
Phylum Rhizobiales:	*Pantoea agglomerans*
Candidatus *Rhizobium massiliae*	*Rhizobium radiobacter*
Rhizobium pusense	*Pseudomonas aeruginosa*
Phylum Enterobacteriales	*Ralstonia pickettii*
Enterobacter ludwigii	*Serratia liquefaciens / grimesii*
Enterobacter mori	*Stenotrophomonas maltophilia*
Phylum Pseudomonales:	*Bacillus pumilus / safensis*
Pseudomonas guariconensis	*Klebsiella oxytoca*
Pseudomonas mohnii	*Pantoea ananatis*
Pseudomonas plecoglossicida	*Pseudomonas fluorescens*
Phylum Xanthomanadales:	*Pseudomonas plecoglossicida*
Stenotrophomonas rhizophila	*Pseudomonas tolaasii*
	Bacillus marisflavi
(Ottesen *et al*. 2013)	*Brachybacterium alimentarium*
Genera: *Pseudomonas*, *Micrococcineae*,	*Chryseobacterium culicis*
Xanthomonas, *Methylobacterium*,	*Empedobacter brevis*
Rhizobium, *Sphingomonas*	*Escherichia vulneris*
	Pseudomonas marginalis
	Acinetobacter guillouiae
	Brachybacterium phenoliresistens
	Burkholderia pyrrocinia / cepacia
	Curtobacterium flaccumfaciens
	Enterobacter cancerogenus
	Microbacterium saperdae
	Pantoea eucrina
	Providencia alcalifaciens
	Pseudomonas alcaligenes
	Pseudomonas cichorii
	Pseudomonas corrugata
	Serratia rubidaea
	Sphingobacterium multivorum
	Brevibacterium epidermidis
	Enterobacter cloacae
	Acinetobacter baumannii / pittii
	Acinetobacter gyllenbergii
	Aeromonas caviae
	Arthrobacter globiformis
	Bacillus oleronius
	Bacillus ruris
	Brachybacterium rhamnosum
	Brevibacterium casei
	Clavibacter michiganensis subsp. *michiganensis*
	Enterobacter aerogenes (*Klebsiella mobilis*)
	Enterobacter nimipressuralis
	Exiguobacterium undae
	Klebsiella variicola
	Kluyvera intermedia
	Paenibacillus xylanilyticus
	Raoultella planticola / ornithinolytica
	Sphingomonas paucimobilis
	Staphylococcus epidermidis
	Staphylococcus pasteuri
	Staphylococcus sciuri subsp. *sciuri*
	Staphylococcus warneri
	Tetragenococcus halophilus subsp. *halophilus*
	Xanthomonas axonopodis

Table 10. Comparison of the species composition of healthy tomato microbiome with a list of bacterial species isolated from tomato plant tissues examined in the Plant Disease Clinic in 2013–2019 – continuation

Healthy tomato microbiome according to literature	Bacterial species identified in tomato tissue samples tested in Plant Disease Clinic
	Bacillus atrophaeus / subtilis
	Acinetobacter beijerinckii
	Acinetobacter genomospecies
	Acinetobacter johnsonii
	Aeromonas hydrophila subsp. *anaerogenes*
	Aeromonas hydrophila subsp. *hydrophila*
	Arthrobacter histidinolovorans
	Arthrobacter woluwensis
	Bacillus amyloliquefaciens subsp. *amyloliquefaciens*
	Bacillus indicus
	Bacillus nealsonii
	Bacillus safensis
	Bordetella trematum
	Brevibacterium sanguinis
	Burkholderia ambifaria / cepacia
	Cedecea neteri
	Chryseobacterium balustinum
	Chryseobacterium scophthalmum
	Chryseobacterium taichungense
	Citrobacter freundii
	Citrobacter sedlakii
	Citrobacter werkmanii
	Clavibacter michiganensis subsp. *tessellarius*
	Comamonas testosteroni
	Corynebacterium kroppenstedtii
	Cupriavidus gilardii
	Cupriavidus pauculus
	Curtobacterium flaccumfaciens pv. *oortii*
	Curtobacterium luteum
	Dermacoccus nishinomiyaensis
	Enterobacter amnigenus
	Erwinia rhapontici
	Flavobacterium johnsoniae
	Janibacter hoylei / anophelis
	Jonesia denitrificans
	Kluyvera ascorbata
	Kocuria varians
	Leclercia adecarboxylata
	Macrococcus brunensis
	Microbacterium arborescens
	Microbacterium flavescens
	Microbacterium testaceum
	Mirobacterium maritypicum
	Moraxella lincolnii
	Novosphingobium capsulatum
	Ochrobactrum anthropi
	Ochrobactrum grignonense
	Paenibacillus alkaliterrae
	Paenibacillus anaericanus
	Paenibacillus provencensis
	Paenibacillus soli
	Paenibacillus stellifer
	Paenibacillus taichungensis / pabuli
	Paenibacullus popiliae
	Pantoea ananatis pv. *ananatis*
	Pantoea cypripedii

Table 10. Comparison of the species composition of healthy tomato microbiome with a list of bacterial species isolated from tomato plant tissues examined in the Plant Disease Clinic in 2013–2019 – continuation

Healthy tomato microbiome according to literature	Bacterial species identified in tomato tissue samples tested in Plant Disease Clinic
	Paraburkholderia caryophylli
	Pectobacterium atrosepticum
	Pediococcus acidilactici
	Providencia stuartii
	Pseudomonas asplenii
	Pseudomonas caricapapayae
	Pseudomonas flavescens
	Pseudomonas fragi
	Pseudomonas fulva
	Pseudomonas nitroreducens
	Pseudomonas oleovorans subsp. *oleovorans*
	Pseudomonas stutzeri
	Pseudomonas synxantha
	Pseudomonas syringae
	Pseudomonas syringae pv. *daphniphylli*
	Pseudomonas syringae pv. *helianthi*
	Pseudomonas syringae pv. *maculicola*
	Rathayibacter rathayi
	Rothia amarae
	Staphylococcus capitis subsp. *urealyticus*
	Staphylococcus cohnii subsp. *cohnii*
	Staphylococcus intermedius
	Staphylococcus sciuri subsp. *carnaticus*
	Staphylococcus xylosus
	Streptococcus oralis
	Tatumella punctata
	Xanthomonas hortorum pv. *pelargonii*
	Yersinia intermedia

classical microbiology. Hence, some bacterial species may in fact, not be a part of a healthy tomato plant microbiome. Nevertheless, these data may give insight into the dynamics of disease developmental processes.

In summary, the data presented above represents an approximation of the current biodiversity state of the main crops in Poland, along with the most common bacterial symbionts and pathogens. Also, the data presented in the supplement constitute a good basis for determining the profile of bacterial diseases of agricultural and ornamental plants in Poland. This information is extremely valuable primarily for phytosanitary services, e.g. for the development of control plans and for further research because it includes new data about plant microbiomes.

References

Ahmed F.A., Arif M., Alvarez A.M. 2017. Antibacterial effect of potassium tetraborate tetrahydrate against soft rot disease agent *Pectobacterium carotovorum* in tomato. Frontiers in Microbiology 8: 1–9. DOI: 10.3389/fmicb.2017.01728

Bosmans L., Moerkens R., Wittemans L., De Mot R., Rediers H., Lievens B. 2017. Rhizogenic agrobacteria in hydroponic crops: epidemics, diagnostics and control. Plant Pathology 66: 1043–1053. DOI: https://doi.org/10.1111/ppa.12687

Buell C.R., Joardar V., Lindeberg M. Selengut J., Paulsen I.T., Gwinn M.L. *et al.* 2003. The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. Proceedings of the National Academy of Sciences of the United States of America 100: 10181–10186. DOI: 10.1073/pnas.1731982100

Chojniak J., Jałowiecki Ł., Dorgeloh E. Hegedusova B., Ejhed H., Magnér J., Płaza G. 2015. Application of the BIOLOG system for characterization of *Serratia marcescens* ss *marcescens* isolated from onsite wastewater technology (OSWT). Acta Biochimica Polonica 62: 799–805. DOI: 10.18388/abp.2015_1138

Ciardi J.A., Tieman D.M., Lund S.T., Jones J.B., Stall R.E., Klee H.J. 2000. Response to *Xanthomonas campestris* pv. *vesicatoria* in tomato involves regulation of ethylene receptor gene expression. Plant Physiology 123: 81–92. DOI: 10.1104/pp.123.1.81

Coutinho T.A., Venter S.N. 2009. *Pantoea ananatis*: an unconventional plant pathogen. Molecular Plant Pathology 10: 325–335. DOI: 10.1111/j.1364-3703.2009.00542.x

Daami-Remadi M. 2007. First report of *Pectobacterium carotovorum* subsp. *carotovorum* on tomato plants in Tunisia. Tunisian Journal of Plant Protection 2: 1–5.

Esker P.D., Nutter F.W. 2002. New frontiers in plant disease losses and disease management assessing the risk of stewart's

disease of corn through improved knowledge of the role of the corn flea beetle vector. Phytopathology: 1999–2001.

Freeman N.D., Pataky J.K. 2001. Levels of stewart's wilt resistance necessary to prevent reductions in yield of sweet corn hybrids. Plant Disease 85: 1278–1284. DOI: https://doi.org/10.1094/PDIS.2001.85.12.1278

Gartemann K.H., Kirchner O., Engemann J., Gräfen I., Eichenlaub R., Burger A. 2003. *Clavibacter michiganensis* subsp. *michiganensis*: first steps in the understanding of virulence of a Gram-positive phytopathogenic bacterium. Journal of Biotechnology 106: 179–191. DOI: https://doi.org/10.1016/j.jbiotec.2003.07.011

SP. 2018. Produkcja upraw rolnych i ogrodniczych w 2017 r. Statistics Poland: 1–84.

Iakimova E.T., Sobiczewski P., Michalczuk L., Wegrzynowicz-Lesiak E., Mikiciński A., Woltering E.J. 2013. Morphological and biochemical characterization of *Erwinia amylovora*-induced hypersensitive cell death in apple leaves. Plant Physiology and Biochemistry 63: 292–305. DOI: 10.1016/j.plaphy.2012.12.006

Jones J.B. 1986. Survival of *Xanthomonas campestris* pv. *vesicatoria* in Florida on tomato crop residue, weeds, seeds, and volunteer tomato plants. Phytopathology 76: 430.

Kałużna M., Pulawska J., Waleron M., Sobiczewski P. 2014. The genetic characterization of *Xanthomonas arboricola* pv. *juglandis*, the causal agent of walnut blight in Poland. Plant Pathology 63: 1404–1416. DOI: https://doi.org/10.1111/ppa.12211

Kałużna M., Willems A., Pothier J.F., Ruinelli M., Sobiczewski P., Puławska J. 2016. *Pseudomonas cerasi* sp. *nov*. (non Griffin, 1911) isolated from diseased tissue of cherry. Systematic and Applied Microbiology 39: 370–377. DOI: 10.1016/j.syapm.2016.05.005

Krawczyk K., Borodynko-Filas N. 2020. *Kosakonia cowanii* as the new bacterial pathogen affecting soybean (*Glycine max* Willd.). European Journal of Plant Pathology 157: 173–183. DOI: https://doi.org/10.1007/s10658-020-01998-8

Krawczyk K., Kamasa J., Zwolińska A., Pospieszny H. 2010. First report of *Pantoea ananatis* associated with leaf spot disease of maize in Poland. Journal of Plant Pathology 92: 807–811. DOI: http://dx.doi.org/10.4454/jpp.v92i3.332

Krawczyk K., Łochyńska M. 2020. Identification and characterization of *Pseudomonas syringae* pv. *mori* affecting white mulberry (*Morus alba*) in Poland. European Journal of Plant Pathology 158: 281–291. DOI: https://doi.org/10.1007/s10658-020-02074-x

Krawczyk K., Zwolińska A., Pospieszny H., Borodynko N. 2016. First report of 'Candidatus Phytoplasma asteris'-related strain affecting juniperus plants in Poland. Plant Disease 100: 2521–2521. DOI: https://doi.org/10.1094/PDIS-05-16-0621-PDN

Lukezic F.L. 1979. *Pseudomonas corrugate*, a pathogen of tomato, isolated from symptomless alfalfa roots. Phytopathology 69: 27. DOI: 10.1094/Phyto-69-27

Mansfield J., Genin S., Magori S., Citovsky V., Sriariyanum M., Ronald P., Dow M., Verdier V., Beer S.V., Machado M.A., Toth I., Salmond G., Foster G.D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Molecular Plant Pathology 13: 614–629. DOI: 10.1111/J.1364-3703.2012.00804.X

Mikiciński A., Sobiczewski P., Puławska J., Maciorowski R. 2016. Control of fire blight (*Erwinia amylovora*) by a novel strain 49M of *Pseudomonas graminis* from the phyllosphere of apple (*Malus* spp.). European Journal of Plant Pathology 145: 265–276. DOI: https://doi.org/10.1007/s10658-015-0837-y

Mikiciński A., Sobiczewski P., Sulikowska M., Puławska J., Treder J. 2010. Pectolytic bacteria associated with soft rot of calla lily (*Zantedeschia* spp.) tubers. Journal of Phytopathology 158: 201–209. DOI: https://doi.org/10.1111/j.1439-0434.2009.01597.x

Nabhan S., Boer S.H., De Maiss E., Wydra K. 2019. *Pectobacterium aroidearum* sp. *nov*., a soft rot pathogen with preference for monocotyledonous plants. International Journal of Systematic and Evolutionary Microbiology: 2520–2525. DOI: 10.1099/ijs.0.046011-0

Ottesen A.R., González Peña A., White J.R. Pettengill J.B., Li C., Allard S., Rideout S., Allard M., Hill T., Evans P., Strain E., Musser S., Knight R., Brown E. 2013. Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). BMC Microbiology 13: 114. DOI: https://doi.org/10.1186/1471-2180-13-114

Pospieszny H., Krawczyk K., Kamasa J., Petrzik K. 2007. First report of a phytoplasma affecting tomato in Poland. Plant Disease 91: 1054. DOI: https://doi.org/10.1094/PDIS-91-8-1054B

Pulawska J., Maes M., Willems A., Sobiczewski P. 2000. Phylogenetic analysis of 23S rRNA gene sequences of *Agrobacterium*, *Rhizobium* and *Sinorhizobium* strains. Systematic and Applied Microbiology 23: 238–244. DOI: https://doi.org/10.1016/S0723-2020(00)80010-7

Rapicavoli J., Ingel B., Blanco-Ulate B., Cantu D., Roper C. 2018. *Xylella fastidiosa*: an examination of a re-emerging plant pathogen. Molecular Plant Pathology 19: 786–800. DOI: 10.1111/mpp.12585

Sawada H., Azegami K. 2014. First report of root mat (hairy root) of tomato (*Lycopersicon esculentum*) caused by *Rhizobium radiobacter* harboring cucumopine Ri plasmid in Japan. Japanese Journal of Phytopathology 80: 98–114. DOI: https://doi.org/10.3186/jjphytopath.80.98

Scarlett C.M., Fletcher J.T., Roberts P., Lelliott R.A. 1978. Tomato pith necrosis caused by *Pseudomonas corrugata* n. sp. Annals of Applied Biology 88: 105–114. DOI: https://doi.org/10.1111/j.1744-7348.1978.tb00684.x

Schaad N.W., Jones J.B., Chun W. 2001. Laboratory Guide for the Identification of Plant Pathogenic Bacteria. American Phytopathological Society (APS Press), 373 pp.

Tian B., Zhang C., Ye Y., Wen J., Wu Y., Wang H. 2017. Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome. Agriculture, Ecosystems and Environment 247: 149–156. DOI: https://doi.org/10.1016/j.agee.2017.06.041

Xin X.F., Kvitko B., He S.Y. 2018. *Pseudomonas syringae*: what it takes to be a pathogen. Nature Reviews Microbiology 16: 316–328. DOI: 10.1038/nrmicro.2018.17

Zhao Y., Thilmony R., Bender C.L., Schaller A., He S.Y., Howe G.A. 2003. Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. The Plant Journal 36: 485–499. DOI: 10.1046/j.1365-313x.2003.01895.x

Zwolińska A., Borodynko N., Krawczyk K., Pospieszny H. 2016. First report of aster yellows related phytoplasma affecting sugar beets in Poland. Plant Disease 100: 2158. DOI: https://doi.org/10.1094/PDIS-02-16-0225-PDN

Zwolińska A., Krawczyk K., Klejdysz T., Pospieszny H. 2011. First report of 'Candidatus Phytoplasma asteris' associated with oilseed rape phyllody in Poland. Plant Disease 95: 1475. DOI: https://doi.org/10.1094/PDIS-03-11-0177

Zwolińska A., Krawczyk K., Pospieszny H. 2012. Molecular characterization of stolbur phytoplasma associated with pea plants in Poland. Journal of Phytopathology 160: 317–323. DOI: 10.1111/j.1439-0434.2012.01903.x