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# Species composition of the bacterial population colonizing tomato flowers

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## Abstract

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The pathogenic, epiphytic and endophytic bacterial population colonizing tomato flowers in Bulgaria were heterogeneous, including typical pathogens in the host causative agents of bacterial speck and spot and opportunistic pathogens members of the genus *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Sphingomonas*, *Pantoea*, *Serracia*. In the phase mass flowering, was observed a death of flowers as a result of ring shape necrosis of the stalk caused by the species *Xanthomonas vesicatoria* T pathotype, race T2 and *X. euvesicatoria* PT2. *Pseudomonas syringae* pv. *tomato* R1, *X. vesicatoria* T2 and *X. euvesicatoria* T2 developed symptomless on flowers of healthy plants. *Stenotrophomonas maltophilia* colonized deformed and wilted flowers with ring shapes necrosis. *Acinetobacter baumannii* and *Sphingomonas sanguinis* caused the necrotic areas of the periphery and the base of the sepals. *Pantoea dispersa* and *Pseudomonas putida* formed oval dark brown spots without a chlorotic halo along the pedicels. *Serratia odorifera* occupied the withered flowers, which are with ring shapes necrosis. *A. baumannii* and *S. sanguinis* inhibited and suppress of pathogenic species, causative agents of bacterial speck and spot. The presence of bacterial cells of the species *X. vesicatoria* and *X. euvesicatoria* inside the stigma was indirect infection of tomato seeds. Establishing the species composition of the typical and opportunistic bacteria in the flower microbiota will clarify the mechanism of seed infection.

*Keywords:* tomato; flowers; pathogenic; opportunistic bacteria

## Introduction

Bacterial spot and speck of tomato could be some of the most serious and destructive diseases and occur worldwide (Bogatzevska, 2002; Mansfiel et al., 2012; OEPP/EPPO, 2013; Potnis et al., 2015; Timilsina et al., 2015; Wang et al., 2018). The foliar bacterial plant pathogens in Bulgaria were species of the genus *Xanthomonas* (*Xanthomonas vesicatoria, X. euvesicatoria, X. cynarae* pv. *gardneri*) and *Pseudomonas syringae* pv. *tomato* (Bogatzevska, 2002; Kizheva et al., 2013; Stoyanova et al., 2014; Aleksandrova, 2016; Kizheva et al., 2018, 2020; Bogatzevska et al., 2020). Dynamic changes of the species and races composition, polycyclic of the pathogens development symptoms and symptomless in host and non-host, relationship with the microbiota community of *Solanum lycopersicum,* their ability to be stored in a hypobiotic state in the seeds and multiply and reproduce again were a essential for a thorough study of the ethiology of species and the population structure of plant pathogenic bacteria from an ecological and adaptive perspective.

*P. syringae* pv. *tomato*, *X. vesicatoria* and *X. euvesicatoria* developed epiphytically and endophytically throughout the vegetation on leaves, buds (leaf and flower buds), green fruits and were resident on non-host weeds. Epiphytic and endophytic pathogenic population was heterogeneous by species: *P. syringae* pv. *tomato, X. vesicatoria, X. euves-*

*icatoria, P. syringae, P. viridiflava, P. putida* and distributed unevenly in the vegetative and generative organs. The highest concentration of bacterial cells was in the flower buds (Bogatzevska, 2002; Ottesen et al., 2013; Dutta et al., 2014; Potnis et al., 2015; An et al., 2019).

Plant microbiomes were dynamic and undergo succession changes with plant development, possibly with new introductions occurring throughout the plant life cycle. Unique bacterial phylotypes (at 95% identity) were associated with fruits and tomato flowers plants that were not detected in other parts of the plant. These included *Microvirga, Pseudomonas, Sphingomonas, Brachybacterium, Rhizobiales, Paracocccus, Chryseomonas, Microbacterium, Methylobacterium.* The most frequently encountered bacterial taxa across aerial plant regions were *Pseudomonas* and *Xanthomonas* (Ottesen et al., 2013).

Microbial communities of the flowers (anthosphere) were distinct from those of the leaves (phyllosphere), roots (rhizosphere), soil, and pollinators, although all may share many members. Floral microbes (bacteria and fungi) can be endophytic or epiphytic and can be transferred horizontally among flowers by pollinators, wind or rain, or vertically between plant and seed (Rebolleda Gomez et al., 2019). The bacteria were sprayed onto the parent flowers, enter the plant and colonize the emerging seeds (Mitter et al., 2017). Future analyses with additional bio-geographical data set of *S. lycopersicum* microflora will help to identify whether or not a "core" microbiome can be ascribed to tomato and if native flora serve as point source contamination or in an ecologically supportive capacity in the flow of pathogens through an agricultural environment (Ottesen et al., 2013). Therefore, understanding what role, if any, the flower microbiota had in plant reproductive success was important in a variety of contexts, including agriculture, food safety, and the conservation or restoration of native plant communities (Aleklett et al., 2013).

In this work, were presented results of the research species composition pathogenic microbiota, epiphytic and endophytic flower bacterial community colonizing the tomato.

## Material and Methods

***Plant material and isolation***

The bacteria were isolated by the method of serial dilution of infected plant material from flower, pedicels, sepals, petals of visibly healthy and with typical bacterial spot and speck symptoms on the leaves, stems and fruits of tomato plants (varieties with red and pink pigmentation fruits, local accessions, selection materials) from the region of Western Bulgaria (Kostinbrod, Bozhurishte, Vranje, Institute of Plant Physiology and Genetics – IPPG – Sofia) and Southern Bulgaria ("Maritsa" Vegetable crop research institute – Plovdiv (MVCRI), Sadovo, Svilengrad, Pazardzhik) on King's B medium and JDC (Rudolph et al. 1990). Single colonies of white fluorescent, non-fluorescent, yellow and yellow-orange bacteria and pure cultures were stored at 4°C on potato-sucrose agar (PSA).

***Pathogenicity test***

The pathogenic potential of the bacterial strains was examined by infiltration of tobacco cv. Samsun NN (Klement, 1963). Hypersensitive reaction (HR) of tobacco leaves was observed on 18, 24, 36, 72, 96 h and artificial inoculation of tomato plants by vacuum-infiltration method (cv. Milyana, Neven, Kopnezh) (Bogatzevska, 2002). Infiltration was carried out with a vacuum pump (pressure 55-60 kPa; 1at = 101.3 kPa).

The concentration of the bacterial suspension was determined on the McFarland scale with BaCl 2 (Klement et al., 1990).

The symptoms of the test plants typical and untypical for bacterial spot and speck were recorded 4-5 days after the infiltration on the following scale: + from 1 to 3 spots per plant; + + from 4 – 10 spots per plant; + + + from 11 to 16 stains per plant; + + + + over 16 spots per plant (Table 1).

***Phenotypic identification***

The main physiological and biochemical characteristics were determined for the genus differentiation of the pathogenic isolates: Gram reaction, anaerobic growth, and synthesis of fluorescent pigment of King's B medium, formation of yellow or orange pigment colonies on the YDC, catalase and oxidase activity, starch hydrolysis, growth in 3% solution of NaCl. The form and size of the single colonies was characterised on a PSA after incubation 24h at T $28^0$ C (Schaad et al., 2001). Oxidase activity was determined on the standard test strips Bactident® Oxidase (Merck Cat № 1.13300.0001), and the catalase – Bactident® Catalase (Merck Cat № 1.11351.).

***Identification and characterization of the pathogenic isolates of flowers with Biolog™ (GN 2 microplates)***

Identification of the isolates was confirmed by the miniaturized identification system Biolog ™ (Biolog ™, USA) with GN2 MicroPlate™ test plates. The v4.20.05 of the software program MicroLog™ (Biolog ™, USA) was used.

The type strains *X. euvesicatoria* NBIMCC 8731, *X. gardneri* NBIMCC 8730*, X. perforans* NBIMCC 8729), *X. vesicatoria* NBIMCC 2427, *P. syringae* pv. *tomato* ICMP2844, *Sphingomonas sanguinis* ATCC51382 were used as controls, and references strain – *Stenotrophomonas maltophilia* NCBI

**Table 1. Isolations from flowers of local accessions, selection materials and varieties of tomato in 2014–2019**

| № Strains | Location | Plant material | Gram | Oxid | Pathogenicity | | Identification by Biolog™-GN2 |
|---|---|---|---|---|---|---|---|
| | | | | | HR tabaco | Tomato | species,pathotype, race |
| Flower withered | | | | | | | |
| 1 | MVCRI | Sel.mat. | – | – | + | ++ | Serracia odorifera |
| 2 | MVCRI | Sel.mat. | – | – | + | ++ | Serracia odorifera |
| 3 | Kd | Local access. | – | + | + | ++++ | Stenotrophomonas maltophilia |
| 4 | Kd | Local access. | – | + | + | ++++ | Stenotrophomonas maltophilia |
| Bacterial exudate on the flower* | | | | | | | |
| 5. | Pd | Sel. mat. | – | + | – | + | Pseudomonas synxantha |
| 6. | MVCRI | Sel.mat. | – | +/– | + | ++ | *Pseudomonas fluorescens* F |
| 7 | Kd | Local access. | – | + | +/– | ++ | Pseudomonas fluorescens G |
| 8 | Bojuriste | cv. Bela | – | + | +/– | ++ | Pseudomonas fluorescens G |
| 9 | Kd | cv. Milyana | – | + | – | + | *Pseudomonas fluorescens* F |
| Bacterial exudate on the stigma** | | | | | | | |
| 10 | Sadovo | Local access. | – | – | + | +++ | Pseudomonas viridiflava |
| 11 | Kd | cv. Milyana | – | – | + | +++ | Pseudomonas viridiflava |
| Bacterial exudate inside the stigma** | | | | | | | |
| 12 | IPPG | Sel. mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 13 | MVCRI | Sel. mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T3 |
| 14 | Bojuriste | Local access. | – | – | + | ++++ | *Xanthomonas euvesicatoria* T3 |
| 15 | Kd | cv. Milyana | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 16 | Kd | Local access. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| Petals- health | | | | | | | |
| 17 | MVCRI | cv. Milyana | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 18 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 19 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 20 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT3 |
| Sepals-necrotic spot | | | | | | | |
| 21 | Kd | Local access. | – | – | + | ++++ | *Acinetobacter baumanni* G2 |
| 22 | MVCRI | Local access. | – | – | + | ++ | *Acinetobacter baumanni* G2 |
| 23 | MVCRI | cv. Rozovo sartse | – | – | – | ++ | Sphingomonas sanguinis |
| 24 | MVCRI | Sel.mat. | – | – | – | ++ | Sphingomonas sanguinis |
| 25 | MVCRI | Sel.mat. | – | – | – | + | Sphingomonas sanguinis |
| 26 | MVCRI | Sel.mat. | – | – | – | + | Sphingomonas sanguinis |
| 27 | MVCRI | Sel.mat. | – | – | – | ++ | Sphingomonas sanguinis |
| 28 | MVCRI | Sel.mat. | – | – | – | ++ | Sphingomonas sanguinis |
| 29 | MVCRI | Sel.mat. | – | – | – | ++ | Sphingomonas sanguinis |
| 30 | Kd | Local access. | – | – | – | ++ | Sphingomonas sanguinis |
| 31 | Kd | Local access. | – | – | + | ++++ | *P. syringae* pv. *tomato* R0 |
| 32 | IPPG | Sel.mat. | – | – | – | ++ | Sphingomonas sanguinis |
| 33 | IPPG | Sel.mat. | – | – | – | ++ | Sphingomonas sanguinis |
| 34 | Kd | Local access. | – | – | – | + | Sphingomonas sanguinis |
| 35 | Kd | Local access. | – | – | – | ++ | *Sphingomonas sanguinis* |
| 36 | IPPG | Sel.mat. | – | – | – | ++ | *Sphingomonas sanguinis* |
| 37 | Kd | Local access. | – | – | – | ++ | *Sphingomonas sanguinis* |
| 38 | Kd | cv. Milyana | – | – | – | + | Sphingomonas sanguinis |
| 39 | MVCRI | cv. Rozovo sartse | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 40 | MVCRI | cv. Aleno sartse | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |

**Table 1. Continued...**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 41 | MVCRI | cv. Milyana | – | – | + | ++++ | *Xanthomonas vesicatoria* T1 |
| 42 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T3 |
| 43 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 44 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 45 | MVCRI | cv. Amalia | – | – | + | ++++ | *Xanthomonas vesicatoria* T1 |
| 46 | IPPG | Sel.mat. | – | – | + | ++++ | *P. syringae* pv. *tomato* R1 |
| Flower pedicel- spots | | | | | | | |
| 47 | MVCRI | Sel.mat. | – | –/+ | – | ++ | Pantoea dispersa |
| 48 | IPPG | Sel.mat. | – | –/+ | – | ++ | Pantoea dispersa |
| 49 | Vrana | cv. Trapezitsa | – | + | – | ++ | *Pseudomonas putida* |
| 50 | Pd | Local access. | – | + | – | ++ | *Pseudomonas putida* |
| 51 | Vrana | cv. Trapezitsa | – | – | + | ++++ | *P. syringae* pv. *tomato*-R0 |
| 52 | IPPG | Apedice | – | – | + | ++++ | *P. syringae* pv.*tomato* R0 |
| 53 | Sadovo | cv. Topak | – | – | + | ++++ | *P. syringae* pv. *tomato* -R1 |
| 54 | Bojuriste | cv. Bela | – | – | + | ++++ | *P. syringae* pv. *tomato* R0 |
| 55 | Kd | Local access. | – | – | + | ++++ | *P. syringae* pv. *tomato* R1 |
| Ring shapes necrosis on the pedicel | | | | | | | |
| 56 | Kd | Local access. | – | – | + | ++++ | *Xanthomonas vesicatoria* T3 |
| 57 | Svilengrad | Local access. | – | – | + | ++++ | *Xanthomonas euvesicatoria* T1 |
| 58 | MVCRI | cv. Aleno satrse | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 59 | MVCRI | cv. Aleno sartse | – | – | + | ++++ | *Xanthomonas vesicatoria* T1 |
| 60 | MVCRI | Sel. mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 61 | MVCRI | cv. Rozovo sartse | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 62 | MVCRI | Sel.mat. | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 63 | MVCRI | cv.IZK Alya | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 64 | MVCRI | cv. IZK Alya | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 65 | Sadovo | cv. Kopnezh | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 66 | Pz | cv. Bivolsko sartse | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT2 |
| 67 | Kd | Local access. | – | – | + | ++++ | *Xanthomonas vesicatoria* PT2 |
| 68 | IPPG | Sel.mat. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 69 | Bojuriste | Local access. | – | – | + | ++++ | *Xanthomonas euvesicatoria* T1 |
| 70 | IPPG | Local access. | – | – | + | ++++ | *Xanthomonas euvesicatoria* PT1 |
| Health flowers from healthy plants | | | | | | | |
| 71 | IPPG | Local access. | – | – | + | ++++ | *P. syringae* pv. *tomato* R1 |
| 72 | IPPG | Local access. | – | – | + | ++++ | *P. syringae* pv. *tomato* R1 |
| 73 | Kd | Local access. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 74 | Kd | Local access. | – | – | + | ++++ | *Xanthomonas vesicatoria* T2 |
| 75 | IPPG | Sel.mat. | – | – | + | ++++ | *Xanthomonas euvesicatoria* T2 |
| 76 | IPPG | Sel.mat. | – | – | + | ++++ | *Xanthomonas euvesicatoria* T2 |
| 77 | Vrana | cv. Balkan | – | – | + | ++++ | *Xanthomonas vesicatoria* T3 |
| 78 | Vrana | cv. Trapezitsa | – | – | + | ++++ | *Xanthomonas vesicatoria* T1 |
| | | | | | | | |

Pathogenicity on tomato (cv. Milyana, Neven, Kopnezh): reporting scale: + – from 1 to 3 spots per plant; ++ – from 4 – 10 spots per plant; +++ from 11 to 16 spots per plant; ++++ – over 16 spots per plant; HR – Hypersensitive reaction +; Kd – Kostinbrod; Pd – region Plovdiv; Pz – Pazardzhik; Sel. mat. – selection material; Local access. – Local accessions; Oxid. – oxidase activity; * flowers with annular necrosis of the pedicel;** – healthy flowers;

KU726007 (Stoyanova et al., 2018), *Pseudomonas viridiflava* (Bogatzevska et al., 1992), *P. putida* (Stoyanova et al., 2011), *P. fluorescens* (Stoyanova & Bogatzevska, 2014) (Table 2).

***Differentiation of pathotype and races***

The pathotypes of the 37 strains that caused bacterial spots were defined using tomato cv. Ideal and pepper cv. Ka-

**Table 2. Differences in utilization of Biolog TM GN2 substrates by the Bulgarian strains of genus Xanthomonas isolated from flowers**

| Substrates | *X. vesicatoria* – Bg | | | | *X. euvesicatoria* – Bg | | | |
|---|---|---|---|---|---|---|---|---|
| | Xv* | + | – | **V** | Xeuv* | + | – | **V** |
| Dextrin | + | 21 | | | + | 16 | | – |
| Glycogen | v+ | 21 | | | v– | | 16 | |
| N-acetyl-galactosamine | V | | 21 | | + | 16 | | |
| Cellobiose | V | 19 | | 2 | + | 16 | | |
| D galactose | v– | 18 | 2 | 1 | + | 16 | | |
| Gentibiose | v+ | 19 | | 2 | + | 16 | | |
| α-D-lactose | – | | 21 | | – | | 16 | |
| Lactulose | v– | 17 | 3 | 1 | v | 12 | 3 | 1 |
| Maltose | V | 16 | 3 | 2 | v+ | 16 | | |
| D-Mannitol | V | 4 | 8 | 9 | – | | 16 | |
| Acetic acid | – | | 20 | 1 | v | 1 | 2 | 13 |
| Turanose | V | 3 | 10 | 8 | – | 7 | 2 | 7 |
| *cis*-Aconitic acid | V | | 20 | 1 | v+ | 16 | | |
| α-hydroxybutiric acid | V | | 17 | 4 | v– | | 12 | 4 |
| α-kato butyric acid | V | 3 | 11 | 7 | v– | | 16 | |
| Malonic acid | V | 7 | 9 | 5 | + | 16 | | |
| Propionic acid | V | 6 | 12 | 3 | v | 1 | 6 | 9 |
| D alanine | V | 17 | 2 | 2 | v | 2 | 4 | 10 |
| L-alanine | V | 16 | 2 | 3 | + | 16 | | 0 |
| Asparagine | v– | | 20 | 1 | – | | 16 | |
| L-Glutamic acid | v+ | 21 | | | + | 16 | | |
| Glycyl-L-aspartic acid | v– | | 21 | | – | – | 16 | |
| Glycyl-L-glutamic acid | V | 13 | 3 | 5 | v+ | 10 | 2 | 4 |
| L-proline | V | 1 | 20 | | v | | 16 | |
| L-Threonine | V | 2 | 12 | 7 | v | | 12 | 4 |
| γ-aminobutyric acid | V | | 18 | 3 | – | | 16 | |
| Urocanic acid | V | | 13 | 8 | – | | 16 | |
| Inosine | v– | | 21 | | v | 2 | 10 | 4 |
| Uridine | v– | | 21 | | v | 1 | 9 | 6 |

Xv* Xeuv* – differentiating features for the species by Jones et al. (2000); Stoyanova et al. (2014); + (positive);−(negative).;V (variable).

lifornyisko chudo as test plants. The races of the T (Tomato pathotype -25 strains**)** and PT (pepper tomato pathotype -12 strains) were determined on different tomato genotypes: L Hawaii 7981, L Hawaii 7998 and cv. Ideal (Bogatzevska & Sotirova, 2001, 2002).

The races of the natural pathogenic population of *P. syringae* pv. *tomato* (9 strains) were determined by the method of Bogatzevska et al. (1989). Differentiators tomato cv. Milyana (sensitive) and L Ontario 7710 (resistant to R0).

## Results

***Symptoms on tomato flowers***

In the phase mass flowering on the flower pedicels and the sepals of visibly healthy tomato plants were observed small oval, water soaked, grey – brown spots with a lighter centre and dark periphery. The sepals did not dissolve and were speckled with small necrotic lesions that cover the base or top. Gradually, the tissues burn and desiccate. The flower was underdeveloped. The petals and sepals were covered with small oval, water-soaked lesions, which were gray-brown with dark periphery on the stems and petioles; ring-shape necrosis encompassed petioles and the flower withered or died. The necrotic areas merged to necrotic ring on the flower pedicels, which withered, and the flowers fell off (Figure 1).

From single spots on pedicels, sepals, ring shapes necrosis of the pedicels, bacterial exudate on stigma and withered flowers were isolated 93 fluorescent, non-fluorescent, yellow and yellow-orange greasy pure cultures, pathogenic to tomato

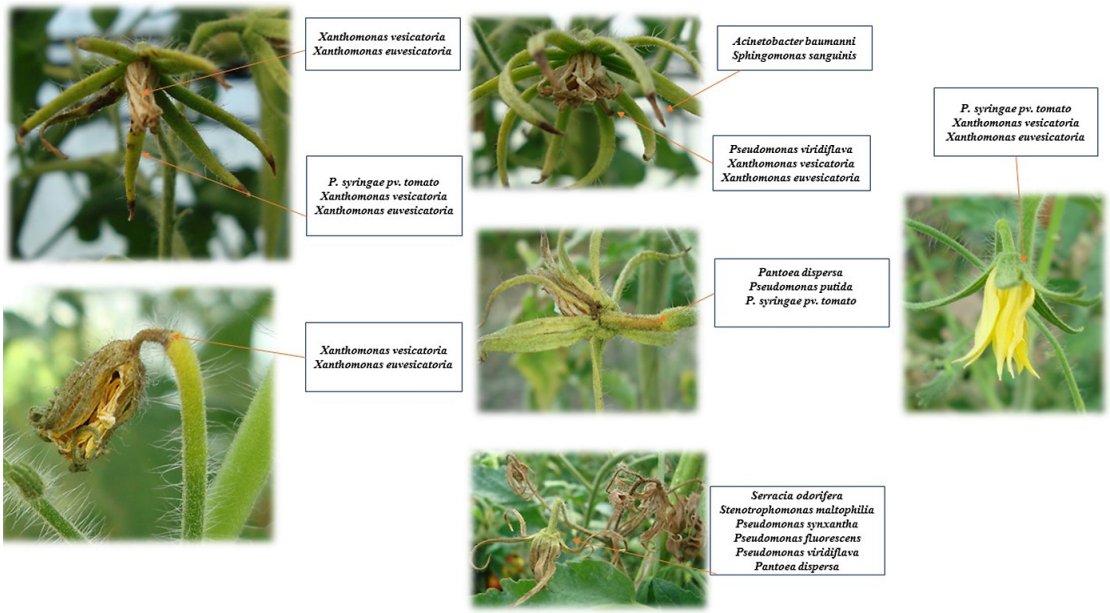


**Fig. 1. Different symptoms from separated flower organs**

were 66 strains, non-pathogenic were 12 isolates; from visibly healthy petals and flowers 15 fluorescent and yellow greasy isolates were obtained pathogenic – 12 strains (Table 1).

***Pathogenicity of isolated strains***

Fluorescent white isolates (No 31**,** 46**,** 51 -55, 71, 72) induced HR in tobacco leaves at 18 h, №10.11 at 36 h, after 96 h № 6, 7, 8. The yellow greasy isolates (37 strains № 11-15, 39-45, 56-70, 73-78) and white non-fluorescent (№ 1, 2, 21, 22) colonies caused HR after 36 h and № 3, 4 after 96 h (Table 1).

The bacteria isolated from the tomato flowers formed various, typical and untypical symptoms of the test plants: 1. *Bacterial spot and speck symptoms*: water soaked, brown spots with a chlorotic halo formed on the leaf isolates with № 31**,** 46**,** 51 -55, 71, 72. Oval, surface lesions, with dark periphery edge and light centre were formed on the leaf stalks and stems. Ring necrosis covered the leaf stalks and the leaf dries. Symptoms were specific for bacterial speck (*P. syringae* pv. *tomato*); small, dark brown spots, single or merged in necrotic areas surrounded by light yellow border cover the leaf lamina of the test plants (cv. Neven, Kopnezh). Irregular, necrotic stripes were formed on the leaf stalks, stems and nerves of the leaves. Symptoms characteristic of the causative agents of bacterial spot (37 isolates) (Table 1). 2. *Untypical symptoms* – fluorescent bacteria: isolates № 49, 50 (*P. putida*) formed single, brown lesions without chlorotic halo and necrotic stripes on the stems of test plants: at *P. synxantha* № 5 – the spots were greasy, with an slightly yellow halo; *P. viridiflava* (№ 10, 11) – small, water soaked, brown-black lesions, with a slight yellow-orange halo damaged to the leaf lamina; greasy, dark-green spots with a faint yellow halo form the bacteria from the group *P. fluorescens* F, G (№. 5, 6, 7, 8) ; non-fluorescent, white, grey, smooth mucoid bacteria: brownish lesions with a very large yellow halo – *Acinetobacter baumannii* (№ 21, 22); dark green, water soak spots of irregular shape, surrounded by a chlorotic halo were formed on the leaves of the test plant (*Stenotrophomonas maltophilia*- № 3, 4); water soak, brownish lesions, chlorosis of tissues in *Serracia odorifera* (№2); yellow bacteria: small, vaguely delineated brownish areas surrounded by chlorosis of the leaf lamina – *Pantoea dispersa* (№ 47, 48); deep yellow orange bacteria: single, small, unformed brown necrotic lesions with chlorosis of tissues, leaves turn yellow and wither (№ 23 to 30, 32-38 – species of genus *Sphingomonas*-15 strains) (Table 1). The other 15 isolates were not pathogenic to tomato.

***Phenotypic identification Biolog ™ GN2***

Patopathogenic and weakly pathogenic bacteria isolated from tomato flowers according to the metabolic profiles on GN2 Biolog ™ were identified as the following species: *X. vesicatoria* 26%, *X. euvesicatoria* 21% (genus *Xanthomonas* 47%); *P. syringae* pv. *tomato* 11% and weakly pathogenic bacteria – *P. putida* 3%, *P. viridiflava* 3%, *P. synxantha*

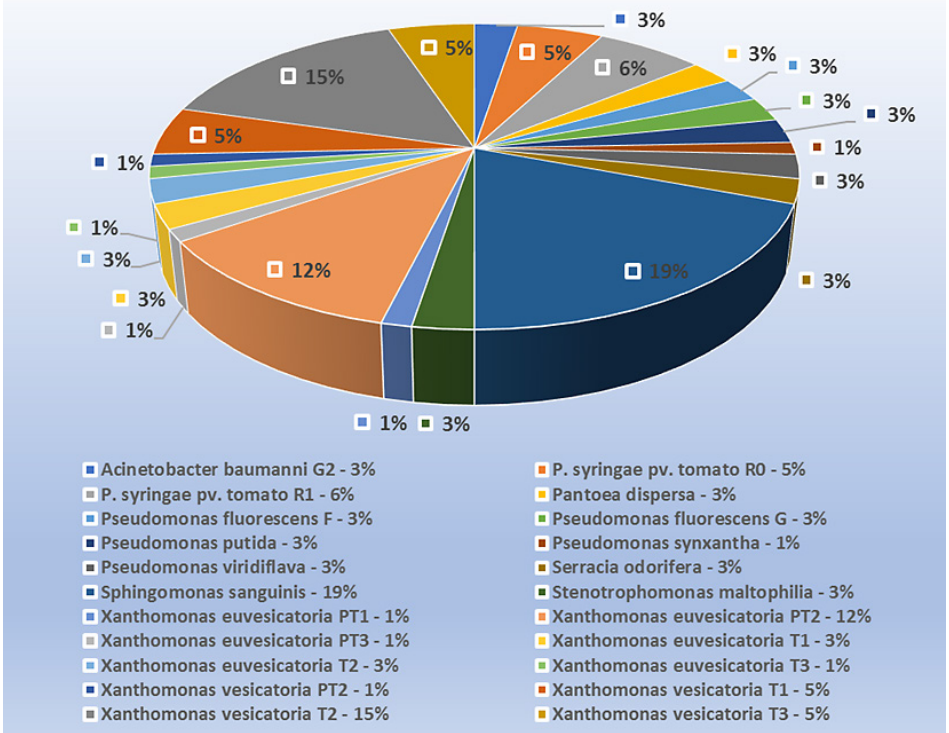


**Fig. 2. Percentage distribution the bacteria species found in infected tomato flowers**

1%, *P. fluorescens* F, G 6% (genus *Pseudomonas* – 24%); *S. sanguinis* 19% (genus *Sphingomonas*-19%); *P. dispersa* 3% (genus *Pantoea*-3%); *S. odorifera* 3% (genus *Serracia*-3%); *A. baumannii* 3% (genus *Acinetobacter*-3%); *St. maltophilia* 3% (genus *Stenotrophomonas*-3%) (Table 1, Figure 2).

***Genus Pseudomonas Migula (1894) (Approved Lists, 1980) emend. Yang et al. (2013)***

The pathogens were Gram-negative, motile rods, catalase positive, synthesizing a fluorescent pigment on King's B medium.

Five types of colonies were distinguished: I. small, greyish, greasy, convex with wrinkled surface and edges; oxidase negative, pathogenic (*P. syringae* pv. *tomato*); II. large, shiny, greasy, dirty white with a hat, oxidase negative, pathogenic isolates (*P. viridiflava*); III. oval-shaped, whole edges, convex, entire, smooth, shiny, non-homogeneous, non-pigmented, thick centred; oxidase positive, weakly pathogenic isolates (*P. putida*); IV. well-formed round protruding colonies, whitish with a slightly wavy surface; oxidase positive, a weakly pathogenic isolates (*P. synxantha*); V. white, smooth, convex, round, greasy shiny with a darker centre, oxidase positive, weakly pathogenic isolates (*P. fluorescens* G, F) (Table 1).

The software of Biolog ™ differentiated five metabolic profiles of pathogenic and the weakly pathogenic fluorescent bacteria and identified the following species: *P. syringae* pv. *tomato, P. viridiflava, P. putida*, *P. synxantha,* and *P. fluorescens* G, F. (Table 1, Figure 2).

***Pseudomonas syringae pv. tomato (Okabe, 1933) Young et al. (1978)***

Pathogenic, fluorescent bacteria were identified as *P. syringae pv. tomato* with probability (Prob) 100%, similarity index (Sim) 0.915-0.988 % and distance index (Dist) 0.18-1.26 %. The type strain (ICMP2844) showed Prob 100%, Sim 0.932 and Dist 1.01. *P. syringae pv. tomato* forms brown, water-soaked lesions with a chlorotic halo, oval on the flower pedicel and small spots, round on the sepals. Developed symptomless in the flowers of healthy plants without symptoms of bacterial speck (Figure 1).

The metabolic profile of the identified flower strains was identical to that of the bacterium isolated from leaves and fruits in tomato-producing areas in Tanzania (Shenge et al., 2008) and Bulgaria (Stoyanova et al., 2015).

The natural epiphytic and pathogenic population of *P. syringae* pv. *tomato* on the tomato flowers consisted of races R0 and R1 (Table 1, Figure 2). Race R1 prevailed in the

symptomless phase of the pathogen from flowers of healthy tomato plants. Metabolic profiles of the race strains R0 and R1 (*P. syringae pv. tomato*) did not differ, which was confirmed by the analysis of Stoyanova et al. (2015).

The pathogenic population of *P. syringae* pv. *tomato* in flowers was homogeneous by phenotypic characteristics and heterogeneous by race composition.

***Pseudomonas viridiflava (Burkholder, 1930; Dowson, 1939)***

The isolates from bacterial exudate on the flower stigma, pathogenic bacteria (Figure 1) were identified as *P. viridiflava* with Prob 100%, Sim 0.713-0.961 and Dist 0.31-2.61. Biolog characteristics of *P. viridiflava* corresponded to that given by Myung et al. (2010), Tsai et al. (2016) and Aleksandrova (2016) of the same pathogen causative agent of: bacterial leaf spot of rape, pith necrosis and necrotic spots on leaf handles and leaf of tomato.

*P. viridiflava* was a polyphagous, typical representative of the epiphytic microflora, an opportunistic and invasive pathogen. It developed in cool and humid weather, epiphytic in tomato and resident in weeds together with *P. syringae* pv. *tomato* and *X. vesicatoria. P. viridiflava* induced yellowing and wilting brown-black spots limited to the pruning sites of the stem, canker of the petioles and pith necrosis on tomato plants (Bogatzevska et al., 1992; Bogatzevska, 2002; Aysan & Uygur, 2005; Tsai et al., 2016; Aleksandrova, 2016).

***Pseudomonas putida (Trevisan, 1889; Migula, 1895)***

The Microlog software identified weakly pathogenic, arginindihydrolase-positive isolates from necrotic spots on the flower pedicel (Figure 1) as the specie *P. putida* with Prob 100%, Sim 0.574-0.608 and Dist 0.398-6.66. Their metabolic profiles were analogous to those identified by Dimartino et al. (2011) and Aleksandrova (2016) on bacteria strains isolated from leaves and leaf petiole of tomato with chlorotic-necrotic spot and soft rots of calla (Stoyanova et al., 2011). *P. putida* was a soil microorganism, that normally did not cause plant disease. The population of bacterium consisted of saprophyte and pathogenic strains for plants, humans and animals. It developed in the rhizosphere of cereal weeds and cultural plants. This pathogen could represent a serious threat for tomato crops grown under salinity stress conditions. *P. putida* and *P. fluorescens* were the causes of tomato pith necrosis (Dimartino et al., 2011).

***Pseudomonas synxantha (Ehrenberg, 1840; Holland, 1920)***

Weakly pathogenic isolates of exudate from the petals of the flowers of a local pink fruit tomato (Figure 1), arginine dihydrolase positive was identified as *P. synxantha* (group of *Pseudomonas fluorescens*) with Prob 96%, Sim 0.590, Dist 5.93. The metabolic profile of the bacteria with this identified by GN2 microplate of isolates of tomatoes (Kůdela et al., 2010; Aleksandrova, 2016).

A strain of *P. synxantha* (DLS A65) active *in vitro* against *X. vesicatoria* was preliminary assayed to control on tomato seeds (Giovanardi et al., 2015). *P. synxantha* inhabited the buds of pine (*Pinus sylvestris* L.), primarily colonizing the cells of scale primordia and resin ducts (Pirttilä et al., 2000).

***Pseudomonas fluorescens (Migula, 1895)***

The BiologTM software identified arginindihydrolase-positive isolates of bacterial exudate on damaged flowers, such as species from the *P. fluorescens* group (Figure 1). The weakly pathogenic strain № 6 was biotype F with Prob 98%, Sim 0.635 Dist 5.35; strain №7, 8, 9 – biotype G with Prob 100%, Sim 0.515 – 0.579, Dist 5.42 – 6.57. Metabolic configuration the strains of *P. fluorescens* tomato isolates, indicated to this species, which was the main and concomitant pathogen of plants forming bulbs (Stoyanova & Bogatzevska, 2014). *P. fluorescens*, alone and in combination with other bacterial species, as causal agent of tomato pith necrosis (Dimartino et al., 2011).

Biolog™ quickly and accurately identified species of the genus *Pseudomonas* and coincided with the analysis of Grimont et al. (1996) using GN 2 plates.

Weakly pathogenic epiphyte and endophyte bacteria *P. viridiflava P. fluorescens, P. putida, P. synxantha* and *S. malthophilia* were isolated from tomato with symptoms of pith necrosis. These strains were opportunistic pathogens and environmental and trophic factors may play a major role in the evolution of bacteria. *P. fluorescens, P. synxantha* and within *P. putida* could be associated with the variety of their habitats (soil, water, plants, meat and dairy products, and animal and human, clinical specimens) (Kůdela et al., 2010; Dimartino et al., 2011).

***Genus Xanthomonas (Dowson, 1939)***

Pathogenic isolates from: dark brown spots with a yellow halo on the sepals, annular necrosis of pedicel, exudate inside the stigma; visibly healthy petals and flowers are Gram – negative, motile rods, catalase positive, formed yellow, greasy colonies (Table 1, Figure 1). Two morphological types were distinguished: I. shiny colonies of drop shape, smooth surface, whole edge, convex with saturated yellow colour (*X. vesicatoria*); II. shinny colonies of round shape such as an egg with a thicker yellow coloured interior and a brighter brim, with a whole edge and a flatter profile (*X. euvesicatoria*).

There were two different metabolic profile groups of strains that were typical of the species: *X. vesicatoria* and *X. euvesicatoria* (Table 2).

***Xanthomonas vesicatoria (ex Doidge, 1920; Vauterin et al., 1995)***

The metabolic characteristic of the 21 strains analysed was not different from the type strain X. *vesicatoria* NBIMCC 2427 and corresponded to the description of the species from OEPP (2013) and Stoyanova et al. (2014). The strains of *X. vesicatoria* isolated from the tomato flowers were strictly amilolytic and did not develop in an environment with *cis* aconitic acid (Figure 1, Table 2).

***Xanthomonas euvesicatoria (Jones et al., 2006)***

The metabolic profile was like that of the type strain *X. euvesicatoria* NBIMCC 8731 and related to the description of the species (OEPP, 2013; Stoyanova et al., 2014). Pathogenic strains of *X. euvesicatoria* develop in an environment with *cis* aconitic acid but were characterised by a diverse response to the starch hydrolysate (5 strains-positive reaction and 11 – negative).

The metabolic profiles of *X. vesicatoria* and *X. euvesicatoria* were distinctly distinguishable (Table 2).

The population of pathogens causing bacterial spot in the tomato flowers was heterogeneous in species, pathotypes and races. The species *X. vesicatoria* and *X. euvesicatoria* were identified (26:21%), two pathotype T and PT (31:15%) were distinguished, the races T1, T2, T3 (9:31:7%), predominant T pathotype (32%) and virulent race T2 (31%) (Tables 1 and 2).

*X. vesicatoria* and *X. euvesicatoria* colonized symptomless flowers, formed necrotic spots by the sepals and annular necrosis along the pedicel of different varieties, selection materials and local accession tomato (Figure 1). The predominant populations of *X. vesicatoria* T race T2. *X. euvesicatoria* PT 2 and *X. vesicatoria* T2 were the causative agents of annular necrosis in the pedicel (Table 1, Figure 1). *X. euvesicatoria* was established for the first time (2015) as a causative agent of bacterial spot on tomatoes in Bulgaria (Aleksandrova, 2016, Kizheva et al., 2020).

Strains belonging to *X. euvesicatoria* and *X. vesicatoria* had a worldwide distribution, *X. vesicatoria* strains primarily infected tomato, while *X. euvesicatoria* pepper. These bacterial populations could also change over time (Timilsina et al., 2015).

***Genus Stenotrophomonas (Palleroni & Bradbury, 1993) gen nov. (Ouattara et al., 2017)***

*Stenotrophomonas maltophilia (Hugh, 1981) Palleroni & Bradbury, 1993, comb.* nov.

Isolates from deformed, wilted flowers (with annular necrosis of the flower pedicel (Figures 1 and 2) were whitish entire-ended colonies, Gram-negative, oxidase-negative, catalase-positive, the strains produced dark brown diffusing pigment on PSA, with a characteristic metabolic profile for the species *S. maltophilia.* They were identified by Prob 100%, Sim. 0.674 – 0.765, Dist. 2.19 – 3.53. Did not differ from the reference strain KU726007 and corresponded to the characteristic of the species (Stoyanova & Bogatzevska, 2012; Mbega et al., 2012; Stoyanova et al., 2018). *S. maltophilia* was plant associated and had been isolated from tomatoes, various weeds, and other plants. The pathogen found in tomato rhizosphere and roots in Mexico (Marquez-Santacruz et al., 2010) and strains isolated from disease tomato fruits and "bald" seeds (Stoyanova & Bogatzevska, 2012; Stoyanova et al., 2018). The strains of *S. maltophilia* were a problem for human medicine with multibuy resistance.

***Genus Sphingomonas Yabuuchi et al. (1990) emend. Feng et al. (2017)***

*Sphingomonas sanguinis (Takeuchi et al., 1993)*

The isolated from top and base necrosis of the sepal's pathogenic bacteria refer to the species *S. sanguinis* (Table 1, Figures 1and 2). Gram-negative, oxidase-negative, catalase-positive with deep yellow orange colonies, formed exopolysaccharide like xanthan (xanthomonads- like bacteria), motile and developed in 3% NaCl. They were identified by Prob 99 – 100%, Sim 0.502 – 0.757, Dist 2.86 – 7.79. Their metabolic profile was analogous to the type strain (ATCC51382) and isolated of tomato seeds in Tanzania (Mbeda et al., 2012). A characteristic feature our strains of *S. sanguinis* was that they very quickly died on nutrient agar, mostly within 2 weeks.

The genus *Sphingomonas* was an aerobic and deep yellow pigment producing bacterium which belongs to the **α**-proteobacteria, opportunistic pathogen. Strains of the genus *Sphingomonas* had a unique characteristic, producing sphingolipids, which differentiated this genus from allied genera (Yabuuchi & Kosako, 2005). *Sphingomonas melonis* sp. new pathogen that caused brown spot on yellow on *Spanish melo*n (*Cucumis melo* var. *inodorus*) fruit (Buonaurio et al., 2002). Xanthomonas-like strains – *S. sanguinis* and *S. terrae* inhabited the epiphytic fruits, flowers and seeds of tomato (Mbeda et al., 2012; Ottesen et al., 2013). *S. sanguinis* and *S. terrae* produced variable black rot symptoms (or brown vein discoloration) on the margins of the inoculated artificial sweet pepper plants, being pathogenic on this host but strains were non-pathogenic on tomato (Mbeda et al., 2012). Bacterial dry rot of mango was caused by *S. sanguinis* in China (Liu et al., 2018).

***Genus Acinetobacter (Brisou & Prévot, 1954) Approved Lists (1980)***

*Acinetobacter baumannii (Bouvet & Grimont, 1986)*

Gram-negative, oxidase-negative, catalase-positive pathogenic bacteria isolated from the sepals of local tomato accessions with pink fruits (Figures 1 and 2) belong to the species *Acinetobacter baumannii*. They were identified by probability 100%, Sim-0.547 – 0.707, Dist 2.67 – 3.53.

Colonies were normally smooth, sometimes mucoid, pale yellow to greyish-white. *A. baumannii* was opportunistic bacteria. The strains of *Acinetobacter* were isolated from the rhizosphere of cultural plants and were known as promote plant growth (produced indole acetic acid phosphate and zinc oxide solubilization, and siderophore), but their use (appendix) was not recommended because individual strains cause human infections.

***Genus Pantoea (Gavini et al., 1989)***

*Pantoea dispersa (Gavini et al., 1989)*

Weakly pathogenic isolated from flowers pedicel of tomato with red fruits referred to the species *Pantoea dispersa* (Table 1, Figures 1 and 2). They were identified by Prob 98%-100%, Sim 0.547 – 0.597, Dis 6.34 – 6.96 (Micrologᵀᴹ 4.20.05).

The genus *Pantoea* was a diverse group of yellow-pigmented, rod-shaped Gram-negative bacteria in the family *Enterobacteriaceae*. They were isolated from soil, water, insects, caused diseases of humans and animals, develop epiphytic and endophytic on different plant species (maize, *sorghum,* cotton, melon and onion). Some *Pantoea* strains produced antimicrobials, and had been developed into commercial biocontrol, and had unique biodegradative capabilities, including metabolic pathways that degrade herbicides and other toxic compounds. *P. dispersa* strains could inhibited the development of black rot disease in sweet potato and sugar cane leaf scald disease as biocontrol agents (Walterson & Stavrinides, 2015). *P. dispersa*, were enough to protect against *P. syringae* pv. *tomato* on tomato seeds (Morella et al., 2019).

***Genus Serratia (Bizio, 1823)***

*Serratia odorifera (Grimont et al., 1978)*

The Biologᵀᴹ software identified the isolates with Prob. 100%, Sim. 0.512 Dist. 7.76 as the type *Serracia odorifera.* Weakly pathogenic bacteria inhabited the withered flowers of the cv. Neven, which were with annular necrosis of the flower pedicel caused by *X. vesicatoria* and *X. euvesicatoria* (Table 1, Figures 1 and 2). Gram-negative bacteria of the genus *Serratia* (family *Enterobacteriaceae*) was isolated from water, air, soil, plants, animals and hospitalized human. They were distributed worldwide *Serratia* species were frequently found associated with plants.

Both species of the family *Enterobacteriaceae* were opportunistic pathogens, which inhabit soil, water, plants, seeds of cereal grasses, sorghum, bulbous caused soft rot.

## Discussion

The species community pathogenic, epiphytic and endophytic that specifically inhabit tomato flowers (selection materials, varieties, local accessions) was heterogeneous, includes typical pathogens in the host causative agents of bacterial speck and spot and opportunistic pathogens members of the genus *Pseudomonas, Stenotrophomonas, Acinetobacter, Sphingomona*s, *Pantoea, Serracia* (Figures 1 and 2). A characteristic special of isolated and identified bacteria was their ability to induce a variety of symptoms along individual plant flower organs and to develop epiphytic and endophytic population (Figure 1).

*X. vesicatoria* (T1, T2, T3), *X. euvesicatoria* (T2, T3; PT2, PT3) and *P. syringae* pv. *tomato* R1 developed symptomless from tomato flowers (Table 1, Figure 1). The inner of the stigma of healthy flowers was inhabited by the species *X. vesicatoria* T2 and *X. euvesicatoria* T3. The natural population of *X. vesicatoria* T2 dominated in flowers without symptoms. *P. syringae* pv. *tomato* R1 colonized symptomless flowers (mass flowering) without visible symptoms of bacterial speck on leaves, stems and fruits (Table 1, Figure 1).

Epiphytic and endophytic population of *X. vesicatoria* reached maximum development in the flower buds, flowers and top leaves. The symptoms of the disease encompass some organs of plants and without symptoms others. *X. vesicatoria* and *P. syringae* pv. *tomato* instigated the characteristic of the bacterial spot and speck of the fruit, and symptomless colonized the buds (leaf and flower) and flowers. *X. vesicatoria* and *P. syringae* pv. *tomato* was developed resident by later spring weeds from the usual association for culture and the region. A major source of infection during the vegetation were tomato plants with a symptomless development of the disease. The causative agents of bacterial speck and spot were stored, survived and distributed with the seeds of tomatoes and resident weed hosts (Bogatzevska, 2002; Duta et al., 2014; Potnis et al., 2015; Kizheva et al., 2018). The infection cycle of *Xanthomonas* and *Pseudomonas syringae* pvs. (bacterial spot of tomato, pepper, bacterial speck) could be divided into the epiphytic and endophytic stage on hosts and non-hosts (Bogatzevska, 2002; An et al., 2019).

Bacterial spot causative agents of tomato and pepper to survived during epiphytic and endophytic growth and to caused disease, considering the role of diverted regulatory

and sensing systems, secreted effectors and the biosynthesis of extracellular polysaccharide and lipopolysaccharides (Bogatzevska, 2002; Ottesen et al., 2013; Romero et al., 2014; An et al., 2019; Rebolleda-Gomez et al., 2019; Schlechter et al., 2019).

Anatomical based analysis in tomato plants also identified *Xanthomonas* (bacterial spot) as an important component of the tomato microbiome. Notably, *Xanthomonas* represented 10% – 40% of the whole bacterial communities of fruits, leaves and flowers (Ottesen et al., 2013). *X. euvesicatoria* inoculated in pepper blossoms led to seed infestation and bacterial spot transmission, were detected from the style that pepper blossoms can be a pathway for seed infestation (Dutta et al., 2014). The presence of bacterial cells of the species *X. vesicatoria* and *X, euvesicoria* inside the stigma was indirect evidence of probable infection of tomato seeds (Table 1, Figure 1).

Epiphytic communities on the exterior of tomato plants played role in the seeding of endophytic communities associated with internal cellular and vascular habitats. Some microbes (*Sphingomona*s, *Pseudomonas, Xanthomonas*) inoculated in flowers, for example, can be transferred to the next generation in the seed. Similarly, epiphytic microbes that attached to persistent floral tissues during fruit development (E. G. styles or sepals) could be transported during seed dispersal. The bacteria were sprayed onto the parent flowers, enter the plant and colonize the emerging seeds (Ottesen et al., 2013; Mitter et al., 2017; Rebolleda-Gomez et al., 2019).

Bacterial spot of tomato was a polycyclic disease. The bacteria pass through the style, enter the ovaries and establish populations that contaminate the seed (Dutta et al., 2014). Flower infection could be carried on to the seeds, direct link between floral infection and inner seed colonisation was established. The bacteria could successfully colonise and caused symptoms in siliques and subsequently colonise both the outer seed coat and the endosperm and embryo. Seedborne-bacterial pathosystems that inoculation of blossoms led to seed infestation within symptomless fruit (Duta et al., 2014; An et al., 2019).

Dynamic changes in the species and differentiation races of the natural populations of bacterial spot, the emergence of new resident hosts, the alternate of the local varieties with unsuitable for soil-climatic conditions of the country, and the international exchange of seeds in recent years led to the emergence of new pathogens, virulent races, unknown opportunistic bacteria in the microbiota of tomato and a change in the symptomatology of the disease.

The ring shape necrosis of the stalk (varieties, selection materials and local accessions with pink fruits) and death of flowers was observed in mass flowering phase (Figure 1). This symptom was caused by the species *X. vesicatoria* and *X. euvesicatoria* in their population dominated T and PT with race T2 (Table 1, Figure 1).

The main causative agent of bacterial spot on tomato (up to 2014 year) in Bulgaria was the species *X. vesicatoria.* During the period 1986-2000 years for highly susceptible Bulgarian varieties tomato (Druzhba, Slava, Ventura, Lira, Mercury). In the flowering phase, rarely sporadically observed symptoms on the pedicel, elliptical, water soak, gray-brown spots, single or mass of lesion, which leads to the wilting of flowers under favourable climatic conditions. *P. syringae* pv. *tomato* was covered pedicel and the sepals with necrotic patches and stripes, surrounded by a chlorotic tissue (Bogatzevska, 2002).

*X. euvesicatoria* PT was established as a causative agent of bacterial spot in pepper for the first time on the territory of the country. The disease was characterized by the defoliation of leaves in the initial phases of development (Bogatzevska et al., 2007). *X. euvesicatoria* was closely specialized in the genome of genus *Capsicum,* as more aggressive on pepper plants and in several countries was the prevalent pathogen (Ignjatov et al., 2010; Hamsa et al., 2010; Vancheva et al., 2014; Vancheva, 2015; Timilsina et al., 2015; Potnis et al., 2015; Vasileva & Bogatzevska, 2019), while *X. vesicatoria* was adapted to the genus *Solanum* and was widely distributed on Bulgaria (Bogatzevska, 2002; Kizheva et al., 2018; Bogatzevska et al., 2020). *X. vesicatoria* and *X. euvesicatoria* caused necrotic ring at the base of the pepper leaf stalk and leaves dropped off. In the primary phenophases, whole plants were defoliated, only the growth top of the plant remains (Bogatzevska et al., 2007; Vancheva, 2015).

Our research showed that the necrotic areas of the periphery and the base of the sepals were caused by the usual epiphytes and endophytes *A. baumannii* and *S. sanguinis.* Typical pathogens for the host were not developed and multiplied in the plant tissues that were suppressed by these species of bacteria. *X. vesicatoria* (T1, T2, T3), *X. euvesicatoria* (PT2) and *P. syringae* pv. *tomato* R0, R1 formed water brown, oval spots with a yellow or chlorotic halo on the surface of the sepals, on which there were necrotic areas at the base and periphery. Probably the presence of *A. baumannii* and *S. sanguinis* inhibited and suppressed of pathogenic species, causative agents of bacterial speck and spot (Figure 1).

*P. dispersa* and *P. putida* formed necrotic elliptical dark brown lesions without a chlorotic halo along the flower pedicels of selection material and local tomato accessions. While *P. syringae* pv. *tomato* R0, R1 developed and multiplied on the flower pedicels, separately formed water, brown strips, surrounded by a chlorotic halo on Bulgarian and introduced varieties (Table 1, Figure 1).

Opportunistic weakly pathogenic bacteria *A. baumannii, S. sanguinis*, *S. odorifera, P. dispersa,* which were in the composition of the tomato flowers microbiota were new species for the Bulgarian phytobacteria science.

*P. dispersa, A. baumannii,* strain of *Sphingomona*s, that occupied floral structures might impose physical barriers to the establishment and proliferation of other microbial taxa, such as pathogens (Rebolleda-Gomez et al., 2019; Morella et al., 2019). Strains of *Sphingomona*s, *Stenotrophomonas*, *Acinetobacter* and some species of *Enterobacteriaceae* specialized in epiphytic and endophytic community on aerial plant organs (seed, leaf and flower). The strains of genus *Acinetobacter, Stenotrophomonas, Sphingomona*s, *Pseudomonas, Xanhomonas* colonized the leaves of tomato epiphytic and endophytic (Ottesen et al., 2013; Rebolleda-Gomez et al., 2019; Morella et al., 2019). *Sphingomonas* was widespread in water, soil, sediments, and in association with 26 plants species belonging to 11 families (Kim et al., 1998; Buonaurio et al., 2002; Costa L.E.O. et al., 2012).

*Acinetobacter* strains playing an important role in plant-growth promotion were used as potential biocontrol agents against *Ralstonia solanacearum-c*ausative agent of wilting on tomatoes (Romero et al., 2014). *S. maltophilia* was a biocontrol role against the soil-borne phytopathogenic fungus *Pythium ultimum in vitro*, *Rhizoctonia solani* of tall fescue (*Festuca arundinacea* Schreb.) and *Ralstonia solanacearum* race 3 biovar 2 the causal agent of potato brown rot (Mbega et al., 2012).

The bacteria were sprayed onto the parent flowers, enter the plant and colonize the emerging seeds. By planting the internally colonized seeds, the bacteria become activated and proliferate and colonize the offspring generation plant, thereby unfolding growth regulation effects from the first day of germination of the offspring crop generation and the relative ease of introducing bacteria into plant seed by applying them on flowers of parent plants the indicates that at least a part of the seed microbiome may derive from flower or pollen colonizing microorganisms and the air or insects visiting the plant during. This aspect had not yet been studied in detail (Mitter et al., 2017).

The role of these tomato interacted microbes and the potential role of bacterial organisms isolated from flowers during this investigated could be explored especially biological control.

## Conclusions

The microbiota colonizing tomato flowers were heterogeneous, including typical pathogens in the host causative agents of bacterial speck and spot and opportunistic pathogens members of the genus *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, *Sphingomonas*, *Pantoea*, *Serracia*.

*Xanthomonas vesicatoria*, X. *euvesicatoria* and *Pseudomonas syringae* pv. *tomato* formed necrotic spots on the sepals, necrosis along the pedicel, colonise symptomless flowers of different varieties, local accessions and selection material.

Species of genus *Sphingomonas* (*S. sanguinis*), genus *Pseudomonas* (*P. putida, P. viridiflava, P. synxantha, P. fluorescens* F, G), *S. odorifera*, *P.dispersa*, *A. baumannii* were weakly pathogenic to tomato, developed epiphytic and endophytic in flowers. Their metabolic profiles were clearly and precisely distinguished by Micrologᵀᴹ 4.20.05.

Ring shape necrosis of the flower pedicel was caused by the species *X. vesicatoria* and *X. euvesicatoria*. The flowers necrotized and withered.

## References

**Aleksandrova, K.** (2016). Resistance of tomato to causal agents of bacterial spot and speck. PhD, Institute of Soil Science, Agrotechnologies and Plant Protection "'Nikola Poushkarov", Sofia, Bulgaria, 148, (Bg).

**Aleklett, K., Hart, M. & Shade, A.** (2014). The microbial ecology of flowers: an emerging frontier in phyllosphere research. *Botany, 92,* 253-266. dx.doi.org/10.1139/cjb-2013-0166.

**An, Q. N., Potnis, M., Dow, F., Vorhölter, Y., He, A., Becker, D., Teper, Y., Li, N., Wang, L., Bleris & Tang, J.** (2019). Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen Xanthomonas. FEMS *Microbiology Reviews, fuz* 024, 1-32. doi: 10.1093/femsre/fuz024 (http://creativecommons.org/licenses/by/4.0/)

**Aysan, Y. & Uygur, S.** (2005). Epiphytic survival of *Pseudomonas viridiflava*, causal agent of pith necrosis of tomato, on weeds in Turkey. *Journal of Plant Pathology, 87 (2),* 135-139.

**Bogatzevska, N.** (2002). Plant pathogenic bacteria from genus *Pseudomonas* group syringae and genus *Xanthomonas* group vesicatoria and axonopodis-phases of the life cycle. Thesis for DSc 362, Institute of Plant Protection, Sofia, Bulgaria, 362, (Bg).

**Bogatzevska, N., Ilieva, E. & Sotirova, V.** (1992). Pith necrosis of greenhouse tomato caused by *Pseudomonas viridiflava* in Bulgaria. *TGC Report, 42,* 11.

**Bogatzevska, N., Stoimenova, E. & Mitrev, S.** (2007). Bacterial and virus diseases spread in Bulgaria and Macedonia on field pepper. *Plant Protection, 18,* 17-21.

**Bogatzevska, N. & Sotirova, V.** (2001-2002). Bacterial spot of tomato in Bulgaria: pathotypes and races. *Genetics and Breeding, 31 (1-2),* 59-66.

**Bogatzevska, N., Sotirova, V. & Stamova, L.** (1989). Race of *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al. *Comp. Rend. Acad. Bulg. Sci., 42 (2),* 129-130.

**Bogatzevska, N., Vancheva, T., Vasileva, K., Kizheva, Y. & Moncheva, P.** (2021). An overview of the diversity of patho-

gens causing bacterial spot on tomato and pepper in Bulgaria. *Bulg. J. Agric. Sci.*, *27 (1),* 137-146.

**Buonaurio, R., Stravato, V., Kosako, Y., Fujiwara, N., Naka, T., Kobayashi, K., Cappelli, C. & Yabuuchi, E.** (2002). *Sphingomonas melonis* sp. nov., a novel pathogen that causes brown spots on yellow Spanish melon fruits. *International Journal of Systematic and Evolutionary Microbiology*, *52,* 2081–2087. http://ijs.sgmjournals.org

**Costa, L. E. O., de Queiroz, M., Borges, A., de Moraes, C. & de Araújo, E.** (2012). Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). *Brazilian Journal of Microbiology, 1 (2),* 1562-1575.

**Dimartino, M., Panebianco, S., Vitale, A., Castello, I., Leonardi, C. & Cirvilleril, G.** (2011). Occurrence and pathogenicity of *Pseudomonas fluorescens* and *P. putida* on tomato plants in Italy. *Journal of Plant Pathology*, *93 (1),* 79-87.

**Dutta, R., Gitaitis, H., Sanders, C., Booth, S. & Smith Langston, D. B.** (2014). Role of blossom colonization in pepper seed infestation by *Xanthomonas euvesicatoria*. *Phytopathology, 104,* 232-239.

**Grimont, P., Vancanneyt, M., Lefevre, M., Vandemeulebroecke, K., Vauterin, L., Brosch, R., Kersters, K. & Grimont, F.** (1996). Ability of Biolog and Biotype-100 systems to reveal the taxonomic diversity of the *Pseudomonads*. *System. Appl. Microbiol., 19,* 510-527.

**Giovanardi, D., Biondi, E., Ignjatov, M., Gašić, K., Ferrari, M., Perez, S., Jevtić, R., Stefani, E.** (2015). Seed transmission of *Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* in tomato and *Xanthomonas euvesicatoria* in pepper and implementation of seed disinfection methods. In: *Mar ci c D, Glavendeki c M, Nicot P, eds. Proceedings of the VII Congress on Plant Protection*. Belgrade, Serbia: Plant Protection Society of Serbia, IOBC-EPRS, IOBC-WPRS, 53 – 8.

**Ignjatov, M., Gašic´,K., Ivanovic´**, M., Ševic´, M., **Obradovic´**, A. **& Miloševic´**, M. (2010). Characterisation of *Xanthomonas euvesicatoria* strains pathogens of pepper in Serbia. *Pesticidi i Fitomedicina*, *25 (2),* 139–149, (Sb).

**Kim, H., Nishiyama, M., Kunito, T., Senoo, K., Kawahara, K., Murakami, K. & Oyaizu, H.** (1998). High population of *Sphingomonas* species on plant surface. *J. Appl. Microbiol., 85,* 731–736.

**Klement, Z.** (1963). Rapid detection of the pathogenicity of phytopathogenic *Pseudomonads*. *Nature*, *199,* 299-300.

**Klement, Z., Mavridis, A. Rudolph, K. & Vidaver, A.** (1990). Inoculation of Plant Tissues. In: *Methods in Phytobacteriology* (eds: Klement et al.) Akademiai Kiado, Budapest, 96 – 121.

**Kizheva, Y., Vancheva, T., Hristova, P., Stoyanova, M., Stojanovska, M., Moncheva, P. & Bogatzevska, N.** (2013). Identification of *Xanthomonas* strains from tomato and pepper and their sensitivity to antibiotics and copper. *Bulg. J. Agric. Sci.*, *19 (2),* 80-82.

**Kizheva, Y., Urshev, Z., Rasheva, I., Vancheva, T., Hristova, P., Bogatzevska, N. & Moncheva, P.** (2018). PFGE: a tool for examination of heterogeneity between the bacterial spot causing xanthomonads of tomato plants in Bulgaria. *Zeutschrift für Naturforschung*, *73 (7-8),* 257-264. https://doi.org/10.1515/znc-2016-0205

**Kizheva, Y., Vancheva-Ebben, T., Hristova, P., Bogatzevska, N. & Moncheva, P.** (2020). First report of *Xanthomonas euvesicatoria* on tomato in Bulgaria. *C. R. Acad. Bulg. Sci.*, *73, (1),* 140-146.

**Kůdela, V., Krejzar, V. & Pánková, I.** (2010). *Pseudomonas corrugata* and *Pseudomonas marginalis* associated with the collapse of tomato plants in rockwool slabhydroponic culture. *Plant Protect. Sci., 46 (1),* 1–11.

**Liu, F., Zhan, R. & He., Z.** (2018). First report of bacterial dry rot of mango caused by *Sphingomonas sanguinis* in China. *Plant Disease, 102(12),* 2632. https://doi.org/10.1094/PDIS-04-18-0589-PDN

**Marquez-Santacruz, H. A., Hernandez-Leon, R., Orozco-Mosqueda, M.C., Velazquez-Sepulveda, I. & Santoyo, G.** (2010). Diversity of bacterial endophytes in roots of Mexican husk tomato plants (*Physalis ixocarpa*) and their detection in the rhizosphere. *Genet. Mol. Res*., *9(4),* 2372-2380.

**Mbega, R., Wulff, G., Mabagala, B., Adriko, J., Lund, S. & Mortensen, N.** (2012). Xanthomonads and other yellow-pigmented *Xanthomonas*-like bacteria associated with tomato seeds in Tanzania. *Afr. J. Biotechnol.,* 11, 14303-14312. http://dx.doi.org/10.5897/AJB12.1305.

**Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Sheibani-Tezerj, R., von Maltzahn, G., Sessitsch, A.** (2017). A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology, 8,* 1- 11. www.frontiersin.org

**Morella, N., Zhang, X. & Koskella, B.** (2019). Tomato seed-associated bacteria confer protection of seedlings against foliar disease caused by *Pseudomonas syringae*. *Phytobiomes Journal, 3*, 177-190. https://doi.org/10.1094/PBIOMES-01-19-0007-R

**Myung, I., Lee, Y., Lee, S., Kim, W. & Shim, H.** (2010). New disease, bacterial leaf spot of rape, caused by atypical *Pseudomonas viridiflava* in South Korea. *Plant Disease, 94(9),* 1164. https://doi.org/10.1094/PDIS-94-9-1164C

**OEPP/EPPO Bulletin** (2013). *43 (1),* 7–20.

**Ottesen A., Peña, A., White, J., Pettengill, J., Li, C., Allard, S., Rideout, St., Allard, M., Hill, Th., Evans, P., Strain, E., Musse, 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),* 1-11.

**Pirttilä, A., Laukkanen, H., Pospiech, H., Myllylä, R. & Hohtola, A.** (2000). Detection of intracellular bacteria in the buds of scotch pine (*Pinus sylvestris* L.) by *in situ* hybridization. *Appl. Environ. Microbiol*., *66,* 7.

**Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E. & Jones, J. B.** (2015). Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology*, *16 (9),* 907–920.

**Rebolleda Gomez, M., Forrester, N., Russell, A., Wei, N., Fetters, A., Stephens, J. & Ashman, T.** (2019). Gazing into the anthosphere: considering how microbes influence floral evolution. *New Phytologist, 224,* 1012–1020. www.newphytologist.com

**Romero, F., Marina, M. & Pieckenstain, F.** (2014). The communities of tomato (*Solanum lycopersicum* L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene pyrosequencing. *FEMS Microbiol. Lett., 351,* 187–194.

**Rudolph, K., Roy, A., Sasser, M., Davis, M., Swings, J. & Gossele, F.** (1990). Isolation of bacteria. In: *Methods in phytobacteriology* (eds. Klement et al ) Akademiai Kiado, Budapest. 45- 86.

**Schlechter, R., Miebac, M. & Remus-Emserman, M.** (2019). Driving factors of epiphytic bacterial communities: A review. *Journal of Advanced Research, 19*, 57–65. http://creativecommons.org/licenses/by-nc-nd/4.0/

**Shenge, C., Stephan, D., Mabagala, R. B., Mortensen, C. N. & Wydra, K.** (2008). Molecular characterization of *Pseudomonas syringae* pv. *tomato* isolates from Tanzania. *Phytoparasitica*, *36,* 338-351.

**Schaad, N. W.** (2001). Initial identification of common genera. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria,* 3rd ed. (N. Schaad, J. Jones, and W. Chun, eds.). APS Press. St. Paul, Minnesota, USA, 1-11.

**Stoyanova, M. & Bogatzevska, N.** (2012). *Stenotrophomonas maltophilia* in scabs of tomato fruits. *Science&Technologies*, *2(6),* 35-38.

**Stoyanova, M. & Bogatzevska, N.** (2014). *Pseudomonas fluorescens* – a primary and secondary pathogen of bulbous plants. *Science & Technologies*, *4(6),* 51-55.

**Stoyanova, M., Aleksandrova, K., Ganeva, D. & Bogatzevska, N.** (2015). Occurrence of *Pseudomonas syringae* pv. *tomato* in Bulgaria. *Agric. Sci. Tech., 7 (1),* 141-144.

**Stoyanova, M., Ganeva, D., Petrov, N. & Bogatzevska, N.** (2018). *Stenotrophomonas maltophilia* – an emerging pathogen on local varieties of tomatoes in Bulgaria. *Acta Microbiologica Bulgarica*, *34 (3),* 180-186.

**Stoyanova, M., Moncheva, P. & Bogatzevska, N.** (2011). New for Bulgaria phytopathogenic bacteria of some ornamental plants. *Scientific Papers of the Jubilee National Scientific Conference with International Participation "The Man and the Universe"*, 6-8 Oct. 2011, Smolyan, Bulgaria, *2*, 735-743.

**Stoyanova, M., Vancheva, T., Moncheva, P. & Bogatzevska, N.** (2014). Differentiation of *Xanthomonas* spp. causing bacterial spot in Bulgaria based on Biolog System. *International Journal of Microbiology*, 7-0. http://dx.doi.org/10.1155/2014/495476.

**Timilsina, S., Jibrin, M. O., Potnis, N., Minsavage, G. V., Kebede, M., Schwartz, A., Bart, R., Staskawicz, B., Boyer, C., Vallad, G. E., Pruvost, O., Jones, J. B. & Goss, E. M.** (2015). Multilocus sequence analysis of xanthomonads causing bacterial spot of tomato and pepper plants reveals strains generated by recombination among species and recent global spread of *Xanthomonas gardneri*. *Appl. Environ. Microbiol., 81,* 1520–1529. doi:10.1128/AEM.03000-14

**Tsai, C. H., Ann, P. J., Lu, Y. S., Chen, M. D., Hwang, S. L. & Hung, T. H.** (2016). Occurrence of pith necrosis of tomato caused by *Pseudomonas viridiflava* in Taiwan. *J. Taiwan Agric. Res., 65(3),* 269–277.

**Vancheva, T.** (2015). Phytopathogenic xanthomonads of pepper plants (*Capsicum annuum*). Ph.D. Thesis. Sofia University "St. Kliment Ohridski", Sofia, Bulgaria, 262, (Bg).

**Vancheva, T., Stoyanova, M., Tatyozova, M., Bogatsevska, N. & Moncheva, P.** (2014). Sub-species diversity of *Xanthomonas euvesicatoria* Bulgarian and Macedonian strains from pepper. *Biotechnology and Biotechnological Equipment*, *28 (4),* 592-601.

**Vasileva, K. & Bogatzevska, N.** (2019). Races of bacterial spot pathogen infecting genus *Capsicum* in Bulgaria. *Agricultural Science and Technology*, *11 (2),* 113-117.

**Walterson, A. & Stavrinides, J.** (2015). *Pantoea*: insights into a highly versatile and diverse genus within the *Enterobacteriaceae*. *FEMS Microbiology Reviews, 39,* 6.

**Wang, Y., Zhang, Y., Gao, Z. & Yang, W.** (2018). Breeding for Resistance to Tomato Bacterial Diseases in China: Challenges and Prospects. *Horticultural Plant Journal*, *4 (5),* 193–207. http://www.journals.elsevier.com/horticultural-plant-journal

**Yabuuchi, E. & Kosako, Y.** (2005). Genus I. *Sphingomonas* Yabuuchi et al. (1990) VP. Validation List no. 34, 321, 1990: 233-287. In: *Bergey's Manual of Systematic Bacteriology,* 2nd edn, vol. 2. Edited by G. M. Garrity. New York: *Springer* 34, 321, 1990: 233-287.

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