Plant Archives Vol. 23, No. 2, 2023 pp. 468-490 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2023.v23.no2.077

A COMPREHENSIVE REVIEW ON RECENT ADVANCES IN THE RESEARCH ON BACTERIAL LEAF SPOT IN TOMATO CAUSED BY *XANTHOMONAS* SPECIES

Shankara Somashetty[1], Srinivasulu[2], Keshavamurhty[3], Roshan Akbarbasha[4], Soumya Krishnamurthy[5], Srinivas Chowdappa[4] and Narasimha Murthy Konappa[4]*

[1]Department of Microbiology, Govt. Science College, Nrupathunga University, Bangalore, Karnataka- 560001, India
[2]Department of Botany, Govt. Science College, Nrupathunga University, Bangalore, Karnataka- 560001, India
[3]Department of life Sciences, Acharya Bangalore B School, Bangalore, Karnataka - 560091 India
[4]Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore, Karnataka- 560056, India
[5]Department of Microbiology, Field Marshal K. M. Cariappa College, Kodagu University, University, Madikeri, Karnataka – 571201, India
*Corresponding author Email: n.murthy10@gmail.com
(Date of Receiving : 10-07-2023; Date of Acceptance : 29-09-2023)

ABSTRACT

The bacterial leaf spot (BLS) is a devastating tomato disease affected by the following Xanthomonas species: *X. campestris* pv. *Vesicatoria, X. euvesicatoria, X. vesicatoria, X. perforans*, and *X. gardner*. Dark lesions and yellow halos on the fruits and foliage are symptoms. The BLS pathogens are widespread in subtropical and tropical areas. Pathogens can be detected using a variety of techniques, most notably a mix of classical and molecular methodologies. Conventional techniques rely on microscopic and culture observation, but Polymerase chain reaction (PCR) and multilocus sequence analysis are obtainable. Control of BLS is problematic because of the infections extensive genetic variety, an absence of durable host resistance, and the ineffectiveness of chemical management. Various bicontrol agents, such as bacteriophage, bacteria and fungi have been documented. Including sustained host resistance is an essential component of continuing integrated BLS management. This review involved state of BLS in tomato comprising its prevalence, pathogen profiles, investigative tools, disease control and resistance development in plant.

Keywords **:** Leaf spot, Tomato, Biochemical characterization, Management, Biological control.

Introduction

Tomato (*Solanum lycopersicum L.*), a vegetable crop of the *solanaceae* family is widely grown in countries such as Spain, Peru, South America, Morocco, Mexico, Turkey, India and Italy. In terms of global acreage, tomato is second only to potato among processing crops thereby contributing significantly to the rural employment (FAO, 2005; Maerere *et al.,* 2006). Additionally, thanks to modern irrigation technology, approximately 7500 tomato varieties are grown all year round in all states of India. Being among the highly treasured fresh vegetables in India tomatoes are not only used for fresh table food but also extensively used in a variety of processed products such as juice, ketchup, sauce, canned fruits, puree, paste and so on. Furthermore, the tomatoes are also known to have medicinal properties, with being rich in vitamins A, B, C and minerals it is also considered to promote gastric secretion and purify blood. Tomato, renowned as the "poor man's apple," is the most widely produced vegetable in terms of direct consumption in raw form, inclusion in food recipes, and marketing in manufacturing preparations, resulting in increased global production. Ten countries account for 90% of the total global production with China taking the lead followed by India, United States, Turkey and Egypt. The remainder of the harvest occurs in the southern hemisphere, specifically in Chile, Brazil, and Argentina.

Diseases in tomatoes

These tomatoes are a warm-season crop, plants cannot withstand severe frost. They can be cultivated commercially at temperatures between 18 and 27 °C, although they grow best between 21 and 23 °C. The production and quality of tomato are influenced by a number of biotic and abiotic variables. Diseases are the most difficult biotic factor to control, resulting in decreased plant health and production. Bacterial plant diseases, for example, there are a major problem on crops due to their infection and spread in favourable environmental conditions (Borkar and Yumlembam 2016; Sundin *et al.,* 2016). One of the most enduring and challenging issues with crops around the world is bacterial illness. Bacterial infections significantly affect quality, yield, and ultimately resulting in financial losses. As well as a variety of bacterial diseases affecting various organs can be found in tomato plants, including stem centrum necrosis, Bacterial leaf spot (BLS) fruit mottling (spotting), fruit top rot, watery rot of stems and fruits, bacterial wilt (brown rot) of stems, and root cancer. Wilting of leaves and

whole plant (*R. solanacearum*), bacterial spot (*X. campestris* pv. *vesicatoria*), bacterial canker (*Clavibacter michiganensis* sub sp. *michiganensis),* brown discoloration of vascular system and adventitious root production (*P. solanacearum, P. corrugata*), pith necrosis (*P. corrugata*), stem necrosis, (*P. marginalis, P. viridiflava*) and bacterial speck (*Pseudomonas syringae* pv. tomato). Disease indications seem on leaves and fruits and decrease the total and marketable fruit yield up to 50% (Abbasi *et al.,* 2015). It causes a reduction in productivity by damaging the leaves, causing flowers and fruit to fall prematurely and by increasing sunburnt fruit (Vale *et al.,* 2004). BLS of tomato, affected by four different bacteria were derived from four former DNA homology groups (A, B, C and D) or phenotypically and genotypically distinct of *X.* species such as *X. euvesicatoria* (Jones *et al.,* 2006) (group A, which contains most of the strains previously known as *X.pv.V*or *X. axonopodis* pv. vesicatoria), *X. vesicatoria,* (group B), *X. perforans* (Jones *et al.,* 2006) (group C) and *X. gardneri* (ex Sutic, 1957) (Jones *et al.,* 2006) (group D). Collectively known as xanthomonads is a limiting factor to tomato production worldwide particularly during seasons of high rainfall and temperature (Potnis *et al.,* 2015; Osdaghi *et al*. 2016; Constantin *et al.,* 2016). Amongst four bacterial species, *X. euvesicatoria* and *X. gardneri* strains infect both tomato and pepper, while *X. perforans* strains only infect tomato and *X. vesicatoria* strains generally infect tomato (Schwartz *et al.,* 2015; Newberry *et al*., 2020). Groups A and B are distributed worldwide, whereas distribution (and resulting economic importance) of groups C and D seems to be limited (Jones *et al.,* 2004). The genus Xanthomonas includes various pathogenic species infecting about 400 diverse host plants subsequent in significant losses in economically essential crops worldwide (Schwartz *et al.,* 2015). Diseases caused by *Xanthomonas* spp. frequently begin with contaminated seeds, though pathogens can be transferred to healthy plants through agricultural practises such as pruning and nebulization of beds by rainwater, soil contamination, and possibly by insects (Ryan *et al.,* 2011). The Xanthomonas genus interacts with the host, which can survive in soil, weeds, plant debris, seeds, in contact with insects, and especially in host plants. These modes of existence promote the spread of epidemics (Marcuzzo, 2009). It has the ability to survive in the seeds for more than a year and to survive in a low metabolic state on tomato seeds for months (Kdela Novacky *et al.,* 2002). BLS is a seedborne disease that enters production fieldsand greenhouses on seeds or transplants.

Pathogen

BLS of tomato is caused by the bacterium *X. campestris* pv *vesicatoria* (X.pv.v) (also known as *X. euvesicatoria* and *X. perforans*), which is a gram negative, aerobic bacterium with straight bacilli and a size of 0.4-0.6 x 1.0-1.8 m. The bacterium produces spots on the leaf, fruit, and stem cankers (AL-Saleh, 2011). It moves with a single polar flagellum. The first tomato bacterial spot was discovered in South Africa in 1914. Patel *et al*. in chilli first described BLS disease in India in 1948 from Pune, Maharashtra. BLS infection causes 40-70% nightshade loss (Mbega *et al.,* 2012). It reduces seed germination, causes defoliation, and results in fruit loss in tomatoes (Kebede *et al.,* 2013).

Taxonomy of *X. campestris* pv *vesicatoria*/ Scientific classification, Kingdom: Bacteria, Phylum: Proteobacteria, Class: Gamma proteobacteria, Order: Xanthomonadales, Family: *Xanthomonadaceae,* Genus: *Xanthomonas,* Species: *X. vesicatoria,* Subspecies: *X. vesicatoria,* form: *X. c. pv. vesicatoria.* Scientific name synonyms such as *X. campestris pv. vesicatoria* (ex Doidge 1920;Vauterin *et al.,* 1995), *X. vesicatoria pv. vesicatoria* (Doidge 1920) Dye, 1978, *X. axonopodis pv. vesicatoria* (Vauterin *et al*., 1995), Trinomial name: *X. campestris* pv. *vesicatoria.*

The X.pv.V colonies are round, slimy, shining, yellow, with even borders colonies on nutrient medium, and yellow pigment is characteristic of the pathogen known as *xanthomonadins*, which produces exopolysaccharide xanthan. The optimal temperature for *X.pv.V* colony growth is 28-30°C, with a pH of 6 to 7. The X.pv.v was initially considered a comparatively similar organism based on virulence and bacteriological determinative tests (Dye *et al.,* 1964). However, current findings indicate that this bacterium is divided into two genetically and phenotypically distinct groups, A and B (Stall *et al.,* 1994), which are known as *X. axonopodis* pv. *vesicatoria* and *X. vesicatoria*, respectively (Vauterin *et al.,* 1995). The *X. vesicatoria* that infects tomato has undergone multiple reclassifications (Jones *et al.,* 2000), but was most recently divided into four different species: *X. euvesicatoria* (Xe), *X. vesicatoria* (Xv), *X. perforans* (Xp), and *X. gardneri* (Jones *et al.,* 2004). This pathogen can be dispersed within the field by rain or sprinkler irrigation droplets, clipping of transplants, aerosols, and it can also be spread through contaminated/infected seeds as the primary source of inoculum (Dutta *et al.,* 2014). The pathogen enters the host via stomata, broken trichomes, hydathodes, or insect puncture or wounding (Koike *et al.,* 2007).

Once inside the plant, the bacteria either locally reproduce in the xylem or colonise the intercellular space to spread systemically throughout the plant (Potnis *et al.*, 2015). Eventually, as bacteria deplete the nutrients in diseased tissue, the epidermal layer withers and ruptures, allowing more bacteria to enter. Although effective (Chitarra *et al.,* 2002), immunological techniques like the enzyme linked immunosorbent assay (ELISA) and flow cytometry are nevertheless time-consuming, labor-intensive, and occasionally erroneous (Chitarra *et al.,* 2002: Park *et al*., 2009). Besides, other approach has been to use DNA markers that are useful in DNA hybridization for sensitive pathogen discovery and identification (Park *et al.,* 2004). Molecular methods use genetic information from pathogens to differentiate pathogens at the species level. For pathogenic xanthomonads, the development of specific primers and DNA probes for identification and detection has been described (Park *et al.,* 2004). Certain PCR primer sequences have been reported for detecting the bacterial spot on tomato (Moretti *et al.,* 2009; Park *et al.,* 2009). DNA-DNA hybridization, repetitive extragenic palindromic polymerase chain reaction(Repetitive sequence based PCR, Rep-PCR), amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP) analyses have all been used to distinguish plant pathogenic Xanthomonas spp (Jones *et al.,* 2004; Rademaker *et al.,* 2000). Many plant pathogenic bacteria are identified and classified using partial sequencing of the 16S rDNA gene (Goncalves and Rosato, 2002).However, for several plant pathogenic bacteria, including Pseudomonas and Xanthomonas, the internal transcribed spacer regions of the rDNA gene are highly conserved and cannot be used to differentiate between species or pathovars (Young *et al.,*

2008). Multi locus sequence typing (MLST) based on six housekeeping genes has recently been used to type Xanthomonas spp. pathogenic to tomatoes (Timilsina *et al.,* 2015). Rep-PCR makes use of consensus primers to target short and interspersed repetitive palindromic elements in the bacterial genome (Versalovic *et al.,* 1994). Repetitive extragenic palindromic (REP) elements, enterobacterial repetitive intergenic consensus (ERIC) sequences, and the BOX element have all been recognised so far.

Symptoms

The BLS symptoms include water-soaked lesions on leaves, stems, and fruit that are brown, small, and angular, causing defoliation and direct fruit damage. The pathogen causes small, circular to irregular greasy spots on the leaf, as well as dark lesions surrounding the spots; the pathogen forms a yellow halo around the spots. Dark-green spots become water-soaked as the disease progresses; after a while, the spots change colour from dark-green to purplish-gray, and a black center appears in the centre of these spots (Venette *et al.,* 1996). The leaves of infected plants may appear scorched. The upper sides of the leaf are sunken, with a translucent centre surrounded by a black margin. When there are a lot of spots, the foliage turns yellow and eventually dies, resulting in defoliation of the lower part of the plant. Lesions tend to concentrate on the leaf edges and tips and can grow to be 35 mm in diameter. This tissue is very dry and easily cracks. After a while, infected leaves begin to defoliate. Severe contagion could cause widespread crop damage and significant yield losses (Pernezny *et al.,* 2003). When bacterial spot lesions are cut in half, they produce ooze, which is visible under a microscope.

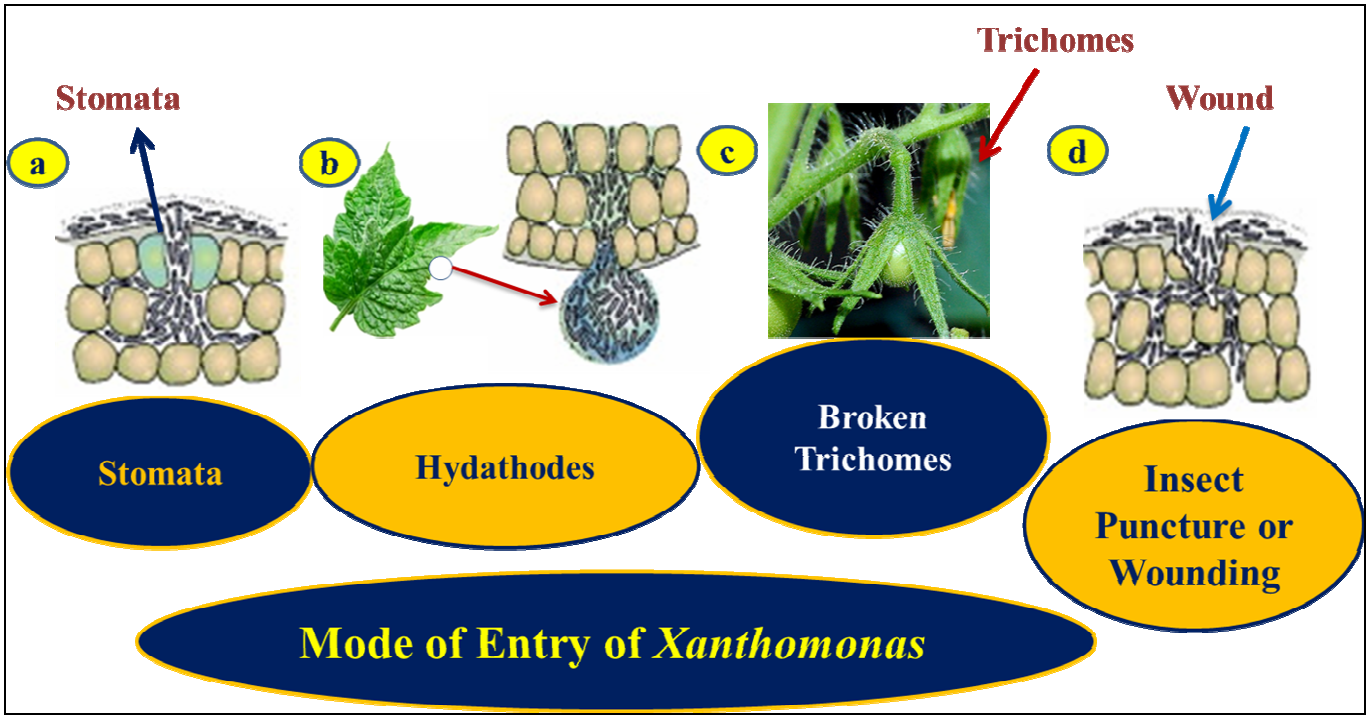

Fig. 1 : Mode of entry of pathogen

Isolation and identification

The isolation of bacteria responsible for leaf spot in tomato leaves, collected from the infected tomato crop was done on nutrient agar media by routine bacteriological laboratory techniques of isolation of plant pathogenic bacteria (Borkar, 2018; Schlechter and Remus-Emsermann, 2019). The surfaces of infected leaves were carefully washed with tap water to eliminate soil debris and then air dried, surface-sterilized in 70% ethanol and then sodium hypochlorite solution 1.25% for 20 s followed by two successive rinses in sterile water. Small pieces of the portion of the leaf spot alongside with the healthy parts was cut using sterile scalpel then teased apart with sterile dissecting needle in 1ml sterile water and allowed to stand for 30 min. Cleaned and surface sterilized leaf sections were blot dried and crushed using sterile motor and pestle in addition to a drop of distilled water forming a bacteria suspension. The resulting suspension was streaked onto modified Tween B media (mTBM) with a sterile bacteriological loop. After 48 h of incubation at 30±2°C; the developed colonies were transferred to NA slants. The plates were then inspected for the presence of bacterial colonies. Pure cultures were obtained by sub culturing characteristic colonies. The media for isolation of X.pv.v was modified Tween B media (mTBM) which was prepared from Peptone 10.0g, Potassium bromide 10.0g, Boric acid 0.10g, Calcium chloride anhydrous 0.25g and Agar 15.00g. 35.35gm of the mixture was weighed and then dissolved in 990ml of sterile water containing 10ml Tween 80 in a conical flask.

The mixture was melted on a hot plate for complete dissolution then autoclaved at 121ºC for 15 minutes at a pressure of 15psi. The media was then cooled to about 45-50°C before adding cephalexin 65mg. Tween B media was modified by using boric acid and cephalexin as inhibitors of contaminants and leaving out cycloheximide, 5-fluorourcil and tobromysin. The pieces of leaf were also placed in sterile cavity blocks and cut to ooze the bacterial pathogen in the sterile distilled water. Twenty ml of the Tween B media (mTBM) medium at 45°C was poured and solidified in petridishes (9 cm size). The bacteria were streaked out with a sterile wire loop on to the plate. The bacterium was identified as X.pv.v based on the identification test (Borkar, 2018).

Isolation and identification of X.pv.v are extraction of bacteria, isolation on selective media, identification of colonies and strain determination. The first step in extraction is preparation of samples and sub-samples. The recommended minimum sample size is 10, 000 seeds, with maximum sub-sample size of 10, 000 seeds according to ISF (International Seed Federation) or at least 1000 seeds according to the EPPO standard. The next step is washing seeds in saline or other solution and by grinding or homogenization. Prepared sample is added to the sterile solution and placed in rotary shaker at 25°C for 1 h.

After extraction the seeds are transferred to the semi-selective media. For semi selective media the following are commonly used: Nutrient medium modified Tween Medium B (mTMB) (McGuire *et al*.,1986), CKTM agar medium (Sijam *et al.,* 1992), nutrient glycerol agar, glucose calcium carbonate agar (GCCA) and Yeast extract dextrose calcium carbonate (YDC) medium, Wilbrink's medium, nutrient agar (NA), nutrient dextrose (ND) agar, YDC agar, nutrient broth yeast extract (NBY) agar, or adenine supplemented yeast peptone glucose agar (YPGA) may also be used. The semi selective medium incubation period lasts 3 to 4 days at 27°C. Colonies form after an incubation period in semiselective medium. Typically, these colonies are yellow, slightly mucoid, mounded, and circular in shape. A white crystalline halo forms around the colonies. The CKTM agar, Modified MXV agar, or Modified TMB agar may allow isolation in samples where high saprophyte populations prevent successful isolation of putative Xanthomonas colonies, but bacterial spot forming Xanthomonas species may not grow equally well on such media.

Identification of bacterial strains

Presumptive X.pv.V colonies and reference strains of the pathogen were identified using several physiological and biochemical characteristics and the hypersensitive reaction on tobacco. In addition, the bacteria were subjected to pathogenicity tests on tomato (a tomato variety with susceptibility to bacterial spot) (Schaad *et al.,* 2001)

Morphological characteristics

Morphological characteristics of isolates of Xanthomonas sp. were observed on the medium growth media. Colonies with mucoid yellow pigmentation were considered positive species of *Xanthomonas.* Typical colonies based on size and colour were recognized, isolated and purified on yeast dextrose calcium carbonate (YDC) to obtain pure culture. On YGCA medium, colonies are bright yellow, circular, with entire margin, wet and shining, mucoid and marginally raised. On other media, colonies appear pale or bright yellow, circular, mucoid and slightly raised. Colony morphology of Xanthomonas strains was detected on diverse semi-selective media such as Tween B (McGuire *et al*. 1986), CKTM (Sijam *et al*. 1992) and SX agar (Shaad and White, 1974), SM agar (Chun and Alvarez, 1983) and modified D-5 agar (MD-5) (Kuan and Minsavage, 1985). On CKTM medium, colonies seem circular, raised, yellow and enclosed by a white crystalline halo. On modified TMB, *Xanthomonas* colonies are yellow, slightly mucous, raised and round. For more purification, individual yellow pigmented colonies are recovered after restreaking on nutrient agar on a non-selective medium. Identification tests of putative *Xanthomonas* spp. should then be carried out. Use of Tween causes a pure halo to form around the yellow colony in 3–7 days. *Xanthomonas* colonies were yellow and mucoid colonies selected and subjected to differential staining (grams stain), for colony morphology determination. A thin smear was prepared on a clean slide from pure colonies of isolates for gram staining.

A drop of normal saline was added to a slide, followed by a loop of pure colony, which was homogenised and air dried by passing over the air. Heat was applied to the airdried slide by rapidly passing it above the flame.After preparing the smear; a drop of primary stain (crystal violet) was added for 1 minute before being rinsed in gently flowing tap water. After that, a drop of Gram's iodine (mordant) was added for 30 seconds before being rinsed off. The cells on the slide were then rinsed after being cleared with 95% ethanol for 30 seconds. To stain gramme negative bacteria, a drop of safranin (counter stain) was applied to the cleared cells on the slide and held for 1 minute before washing away. The slides were blot dried and observed microscopically under SLD inverse light microscope. Gram negative bacteria isolates were identified by their ability to stain pink reddish while those retaining the purple colour of crystal violate were considered Gram positive. Thin smear was set from the colonies, heat fixed by passing the slide above the flame, then methylene blue stained and observed microscopically under oil of emulsion objective lens to reveal the shape and arrangement of the bacterial cells. The cells could be either cocci or bacilli. All the pathogenic strains are Gram negative, non-fluorescent, rod shaped and motile. All strains have oxidative but not fermentative metabolism and a mucous growth on yeast dextrose chalk agar. They made yellow, circular, convex, mucoid colonies on YDC medium, metabolized glucose oxidatively, grew at 37 °C, hydrolyzed gelatin and esculin, produced acids from d-arabinose, d-glucose and mannose, did not reduce nitrates, were oxidase negative and catalase positive, tolerant to 0.02%, but not to 0.1% of triphenyl tetrazolium chloride (TTC). *Xanthomonas vesicatoria* supposed on tomato was confirmed by isolation on semi selective media such as Tween B (McGuire *et al*., 1986) followed by biochemical tests for *Xanthomonas* and virulence tests on tomato.

Biochemical, physiological and serological characterization of the pathogen

The isolates noticeable as gram negative from the loop test were tested using Grams staining. All isolates retained a pinkish color thus confirming that they were gram negative. For the observation of cell size and shape, the counterstained slides were observed using a microscope at 600X magnification. Each slide, representing one isolate, was examined and the shape of the bacteria found to be elongated rods with an average size of 1.04 µm x 0.5 µm (L x W). All isolates tested positive for H_2S production giving a black discoloration on lead acetate paper strips. All the isolates were found catalase positive and gave off H_2O_2 bubbles in the test petri plates. All isolates revealed a yellow color change in test tubes without mineral oil indicating the capacity to oxidize glucose. All the test isolates were oxidase negative and did not display purple color even after 60 sec. All isolates were unable to reduce nitrate to nitrite even after the addition of zinc.

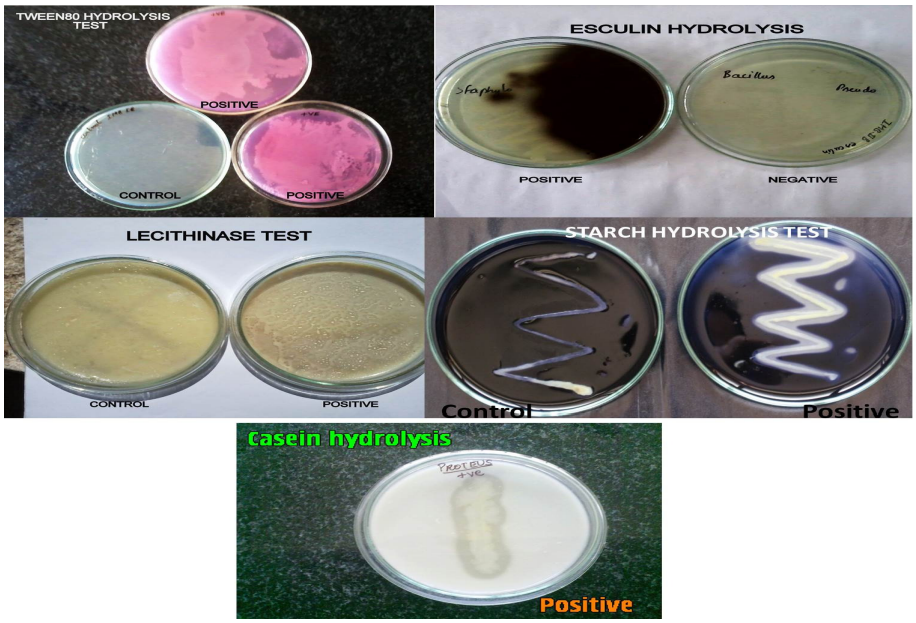

Fig. 2 : Biochemical characterization of the pathogen

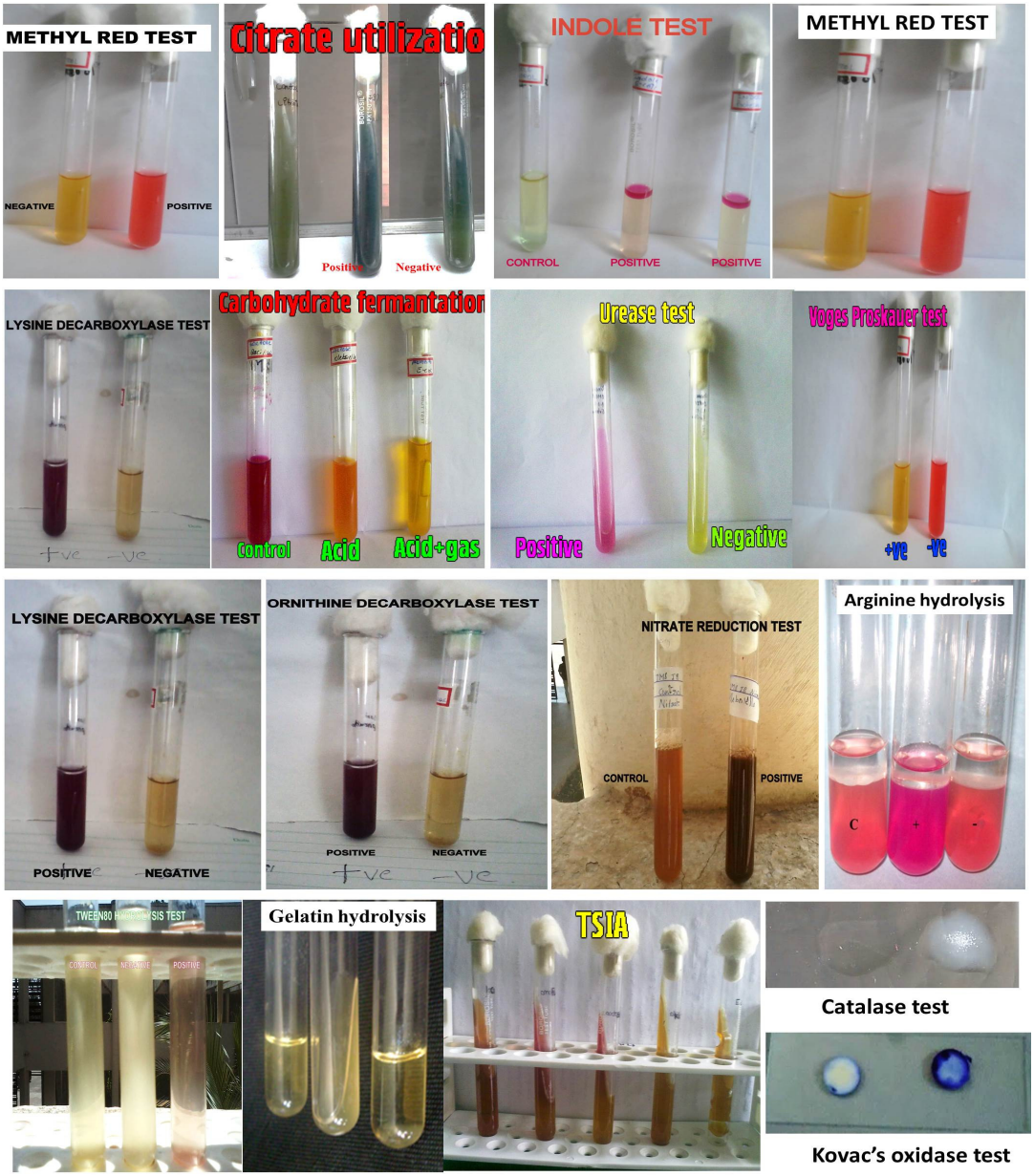

Fig. 3 : Biochemical characterization of the pathogen

Amylolytic and pectolytic activity

Amylolytic activity was tested by rising bacteria containing 0.2% (w/v) soluble starch. Plates were then flooded with Lugol's iodine solution (1% iodine, 2% potassium iodide). A clear zone round the bacterial growth showed hydrolysis of starch (Sands, 1990). For pectolytic activity, bacterial cells were streaked on pectate medium and incubated at 25– 28°C for 2 days. Pectolytic activity for the strains was confirmed by the formation of cloudy halos or dents around the colonies on pectate media plates after incubation of the plates (Keen *et al.,* 1984). All of the *X. vesicatoria* and *X. perforans* reference strains were positive for amylolytic and pectolytic activities.

Carbon source utilization

The resultant pellets were re-suspended in about 20 ml of sterile saline. The suspension was adjusted to an optical density (OD) of 0.3 at 620 nm (corresponding to about 108 cfu ml^{-1}). The GN Microtiter plates were pre-warmed at 28 ± 1°C for about 10 min, before they were inoculated with the *X.pv. vesicatoria* suspension at 150 µl per well, using a pipette. The inoculated plates were then incubated at 28 ± 1°C. Utilization of the carbon source substrates was evaluated visually at 24 and 48 h. Reduction of the tetrazolium dye to violet formazan was recorded as positive for utilization of the compounds. Based on the differences in intensity of the purple colour, the reactions were categorized into four classes as strongly positive (class 3), moderately positive (class 2), borderline (class 1) and negative (0). The strains of *X. pv. vesicatoria* differed extensively in utilization of carbon sources in the Micro Plates. Twenty three carbon sources (D-fructose, -D-glucose, Dmanose, methyl pyruvate, monomethyl succinate, Cisaconitic acid, D-gluconic acid, D, L-lactic acid, bomo succinic acid, D-alanine, L-alanine, L-alanylglycine, Lasparagine, L-aspartic acid, L-glutamic acid, glycyl-Laspartic acid, glycyl-L-glutamic acid, L-histidine hydroxyl L-proline, L-ornithine, Urocanic acid, Inosine, glycerol and glucose-6-phosphate) were utilized by all the *X. pv. vesicatoria* strains. Differences in the carbon source utilization patterns of the *X. pv. Vesicatoria* strains tended to be influenced by geographical origin.

Copper and streptomycin sensitivity

In distilled water, copper sulphate fresh stock solution and streptomycin sulphate were prepared. After autoclaving, the stock solutions were filter sterilised and added to sucrose peptone agar (SPA) at the appropriate concentrations. Tee 24 h NA broth bacterial cultures were serially diluted to a final concentration of 10^3 cfu ml^{-1}. For streptomycin and copper sensitivity, 100µl of the bacterial suspension was plated onto SPA media amended with (20, 50, 100, or 200 g ml^{-1}) streptomycin sulphate, SPA media amended with (100, 200 g ml^{-1}) copper, and SPA media without any copper or streptomycin as a control. Plates were incubated at 28 degrees Celsius for 48 hours and bacterial growth was observed (Obradovic *et al.,* 2004). After 48 h incubation at 28 °C, growth of individual colonies recovered on SPA modified with streptomycin or copper was measured positive for tolerance to streptomycin or copper. Isolates were considered sensitive (S) when they did not grow at with of copper sulphate or streptomycin sulphate and highly resistant (HR) if they grew confluently with each respective chemical. Control cultures of all isolates were incubated in similar conditions in non-amended SPA for comparison purposes and only colonies that presented confluent growth equivalent to that of the controls were considered positive for each respective treatment.

Individual colonies recovered on SPA modified with streptomycin or copper grew positive for streptomycin or copper tolerance after 48 hours incubation at 28 °C. Isolates were classified as sensitive (S) if they did not grow in the presence of copper sulphate or streptomycin sulphate, and highly resistant (HR) if they grew confluently in the presence of both chemicals. Control cultures of all isolates were incubated in non-amended SPA under similar conditions for comparison purposes, and only colonies with confluent growth comparable to the controls were considered positive for each treatment. Maximum strains were produced on SPA medium supplemented with 100 gml^{-1} copper sulphate but not with 200 g ml^{-1}. The strains' growth was inhibited by the lowest concentration of streptomycin sulphate (20 g ml^{-1}). An early copper sensitivity study of *Xanthomonas* spp. causing bacterial spot on processing tomatoes discovered no resistant isolate at 200 g ml^{-1} copper sulphate, but differences in sensitivity at 50 g ml^{-1} copper sulphate.

Antibiotics sensitivity

Polymixin B sulphate, gentamycin sulphate, chloramphenicol, streptomycin sulphate, lincomycin hydrochloride, phosphomycin, bacitracin, vancomycin, ampicillin, and cephazolin were the antibiotics used. Bacterial strains were tested for antibiotic sensitivity by suspending a loopful of 48-hour-old bacterial culture in 20 mL of sterile distilled water. The bacterial suspension was then adjusted to an optical density of 0.06 at 620 nm to 10^8 cfu ml^{-1}, and 10 µl of it was spread on SPA surfaces in Petri dishes. The paper discs were then dipped in the antibiotic solution, placed on the agar surface, and labelled. Inoculated plates were incubated at 28°C for 48 hours and daily inhibition zones around the paper discs were observed. Radial inhibition zones were measured around each disc, corresponding to the level of antibiotic activity for each treatment.

Characterization of races

The plants were tested when they had six leaves. The presence or absence of collapsed tissue at the infiltration site within 24-48 hours after inoculation was used to identify X.pv.v races (Sahin and Miller, 1998). The tomato differences revealed that the strains belonged to at least three different races. We classified the races based on the compatible reactions of the tested genotypes, and the tomato strains belong to tomato race 2.

Serological methods

Tests should be done on seed or plant extracts and their 10 fold dilutions. The use of serological methods in screening seeds or plant extracts would therefore require prior validation of available antibodies against the full range of pathogen diversity to be identified. *X. vesicatoria* strains react differently to the panel of monoclonal antibodies, do not utilize cis-aconitate and have a specific 25- to 27 kDa protein in the SDS-PAGE profile. An indirect enzyme-linked immunosorbent assay (ELISA) was carried out using polyclonal antibodies formed against a tomato strain X.pv.v and alkaline phosphatase conjugated goat anti-rabbit antibodies (Hampton *et al.,* 1990). ELISA based Xcm specific methods that are appropriate for treatment large plant

samples have also been developed for laboratory assesses. ELISA test established serological similarity of the strains and X.pv.v. An indirect enzyme-linked immunosorbent assay (ELISA) was carried out using polyclonal antibodies produced against a tomato strain X.pv.V (NCPPB 422), and alkaline phosphatase conjugated goat anti-rabbit antibodies (SIGMA, A-8025) (Hampton *et al.,* 1990). Also, immunological techniques, such as the ELISA and flow cytometry are relatively insensitive or time consuming than PCR based sensitive assay (Chitarra *et al.,* 2002). Large plant sample-appropriate ELISA-based Xcm-specific procedures have also been developed for laboratory evaluations. The serological resemblance between the strains and X.pv.V was confirmed by an ELISA test. X.pv.v tomato strain polyclonal antibodies (NCPPB 422) and goat anti-rabbit antibodies (SIGMA, A-8025) with alkaline phosphatase conjugation were used in an indirect enzyme-linked immunosorbent assay (ELISA) (Hampton *et al.,* 1990). Additionally, compared to PCR-based sensitivity assays, immunological procedures like the ELISA and flow cytometry are rather insensitive or time-consuming (Chitarra *et al.,* 2002).

Pathogenicity tests

The healthy seed and seedlings underwent tests for pathogenicity on the host plant and other plant species. To verify the outcomes of the virulence assay on the isolated host, bacteria were grown on nutrient agar for a whole night at room temperature. Then, they were suspended in distilled water. The concentration of the bacterial suspensions was measured using a spectrophotometer, and the bacterial suspension was adjusted with sterile distilled water to have an OD 600 value of 0.2 (1×10^8 cfu/ml). Before being sprayed on seedlings or leaf panels that were between the second and fourth leaf stage, 300 µl of inoculum were infused into the suspensions. After being soaked and maintained moist, the inoculated plants were then put in the greenhouse.

Bacterial spot indexes were observed three weeks after inoculation. Water-soaked lesions were classified as susceptible, while tan papery lesions were classified as hypersensitive. Following that, isolates representing each phylogenetic clade were prepared as described above and inoculated to confirm symptomatic pathogenicity. Pin prick, sand paper, and injection were the methods of inoculation used. Paper pins were used to bruise the seeds during the pin prick process, and an inoculum of X.pv.v. (10^8 cfu ml^{-1}) was applied. Sand paper abrasions were made on seeds using sand paper, and the inoculum was applied to the wounded surfaces. In the third method, injection, leaves of young seedlings were inoculated with 1 ml of inoculum using a hypodermic syringe.

Hypersensitivity

The isolated colonies of X.pv.v were confirmed for ability to induce a hypersensitive response on foliage of the non-host plant tobacco (*Nicotiana tabacum*). Each strain was tested for hypersensitivity by infiltrating the bacterial suspension into the intercostal tissue of tobacco leaves (Klement *et al.,* 1964). Test strains were freshly grown on NA at 30±2°C for 24 h and suspended in distilled water, maintaining an inoculum concentration 10^8 cfu ml^{-1}. 1 ml of bacterial suspension was injected into the lower surface of the tobacco leaves and the plants were sealed in plastic bags for 24 h to prevent desiccation. The ability of isolated X.pv.v colonies to induce a hypersensitive response on the foliage of the non-host plant tobacco was confirmed (Nicotiana tabacum). By infiltrating the bacterial suspension into the intercostal tissue of tobacco leaves, each strain was tested for hypersensitivity (Klement *et al*., 1964). Freshly grown on NA at 302°C for 24 hours, test strains were suspended in distilled water with an inoculum concentration of 10^8 cfu ml^{-1}. About 1 mL of bacterial suspension was injected into the lower surface of the tobacco leaves, and the plants were sealed in plastic bags for 24 hours to prevent desiccation. Tobacco seedlings were inoculated with suspensions of the selected isolates at the 5-6 leaf stage. Control plants were injected with sterilized distilled water and kept under same conditions (Klement *et al*., 1964). Necrosis of the infiltrated area after 24 h was considered a positive hypersensitive reaction (HR). At the 5-6 leaf stage, tobacco seedlings were inoculated with suspensions of the selected isolates. Control plants were injected with sterilised distilled water and kept in the same conditions as the experimental plants (Klement *et al*., 1964). A positive hypersensitive reaction was defined as necrosis of the infiltrated area after 24 hours (HR).

Fatty acid methyl ester analysis

Fatty acid methyl ester (FAME) analysis will distinguish *Xanthomonas* spp. isolated from tomato from other bacterial pathogens causing leaf spot symptoms. The strains were grown on Trypticase soy broth agar (TSBA) for 24 hours at 28 °C to examine whole cell fatty acid profiles (Sasser, 1990).

Molecular identification of *Xanthomonas*

The pathogen is identified using biochemical tests and immunological techniques. These methods, however, are time-consuming and produce inconsistent results (Park *et al.,* 2009). Molecular identification of *Xanthomonas* species remains difficult. Dowson (1939) proposed this genus and described 60 species. Bacteria were classified at the time based on phenotypic, biochemical, morphological, and pathogenicity characteristics. Based on DNA:DNA homology and the utilisation of carbon sources (Wang *et al*., 2023). Vauterin *et al*. (1995) recommended the classification of the genus *Xanthomonas* into 20 species, which has been the most widely accepted grouping at the species level. These findings were supported further by rep-PCR and AFLP analyses (Rademaker *et al.,* 2000). Vauterin *et al*. (1995) suggested a reclassification of the xanthomonads and separated *X.pv.V*into two species, with B strains being retained in *X. vesicatoria* and A strains moved to *X. axonopodis* pv. *vesicatoria*. *X. axonopodis* pv. *vesicatoria* was elevated to species and designated *X.* eu*vesicatoria* and two additional xanthomonads related with bacterial spot of tomato were elevated to species and designated as *X.* perforans and *X.* gardneri (Jones *et al.,* 2004). Groups A and C were concisely transferred to *X. vesicatoria*, largely on the basis of DNA homology amongst a large but incomplete collection of xanthomonads (Jones *et al.,* 2000) whereas group B was evidently separated at species level as *X.* vesicatoria. Group D strains, initially identified in the previous Yugoslavia (Sutic, 1957) and containing equal strains from Costa Rica, maintained species status as *X. gardneri* (Jones *et al.,* 2004).

In terms of identification, gene sequencing approaches (DNA barcoding) now provide a relatively simple, lowcost, and robust method for confirming identity and distinguishing the *Xanthomonas* species responsible for bacterial spot. As a

result, these approaches are preferred over the complex and costly DNA: DNA homology analyses, which are not suitable for routine diagnostics. Currently, there are four validly described species of bacterial spot bacteria (*X. vesicatoria, X. euvesicatoria, X. perforans, and X. gardneri*), and *X. axonopodis* pv. *vesicatoria* is no longer a valid name. PCR followed by restriction enzyme analyses and rep-PCR were used to identify Xanthomonas species containing tomato bacterial spot agents (Leite Jr *et al.,* 1995; Louws *et al.,* 1995). There are two conservative duplexPCR tests available to differentiate the four described species: *X. euvesicatoria, X. vesicatoria, X. gardneri,* and *X. perforans*. An earlier AFLP study identified a specific marker for all four species, emphasising the importance of testing a wider range of strains for primer validation (Koenraadt *et al.,* 2009). Based on the sequence data of these markers, four primer combinations have been developed, Bs-XeF/Bs-XeR, Bs-XvF/Bs-XvR, Bs-XgF/Bs-XgR, and Bs-XpF/BsXpR, each amplifying aspecific fragment of each of the four species (Koenraadt *et al.,* 2009). These specific primers seemed intriguing, given that all four species have been reported to occur in tomato-producing areas (Quezado-Duval *et al*., 2005).

The amplification takes place in two distinct reactions, each with two primer combinations (duplex-PCR tests). In addition, as an internal control, the primer combination BAC16-F/ BAC16-R is used in each amplification to generate a 466bp fragment of the 16S rRNA gene. Recent phylogenetic analyses based on DNA sequence similarity between single and multiple gene loci support three distinct species, with *X. euvesicatoria* and *X. perforans* being closely related to each other as well as the recently designated *X. alfalfa* (Hamza *et al.,* 2010). The *X. vesicatoria* species appears to be welldefined; many Xanthomonad pathovars are described for the X. euvesicatoria and *X. hortorum* species level clades. Whole genome sequences from reference strains of *X. euvesicatoria*, *X. perforans*, *X. gardneri*, and *X. vesicatoria* show significant diversity among these pathogens and are identifying genes specific to pepper pathogens as well as other strain specific genes, which may help to explain differences in virulence, aggressiveness, and host preference (Potnis *et al.,* 2011).

Polymerase chain reaction methods

Aqueous suspensions of presumptive isolates comprising approximately 10^6 cfu ml^{-1} can be identified using a series of conventional PCR tests as described (Koenraadt *et al.,* 2009). Sequences, target species and predictable PCR products were as follows, BS-XeF (5′-CATGAAGAACTCGGCGTATCG-3′) and BS-XeR (5′-GTCGGACATAGTGGACACATAC-3′), 173bp for *X. euvesicatoria*, BS-XvF (5′-CC ATGTGCCGTTGAAATACTTG-3′) and BS-XvR (5′-ACAAGAGATGTTGCTATGATTTGC- 3′), 138bp for *X. vesicatoria*, BS-XpF (5′-GTCGTGTTGATGGAGCGTTC-3′) and BS-XpR (5′-GTGCGAGTCAATTAT-CAGAATGTGG- 3′) 197bp for *X. perforans*, and BS-XgF (5′-TCAGTGCT TAGTT-CCTCATTGTC-3′) and BS-XgR (5′-TGACCGA TAAAGACTGCGAAAG-3′), 154-bp amplicon for *X. gardneri*. 16s rRNA internal control BAC16S-F: 5′-TCC TAC GGG AGG CAG CAG T-3′ BAC16S-R (5′-GGA CTA CCA GGG TAT CTA ATC CTG TT-3′). Amplicon sizes in base pairs: Bs-XeF/R primers 173 bp, Bs-XvF/R primers 138 bp, Bs-XpF/R primers 197 bp, Bs-XgF/R primers 154bp, BAC16S-F/R (internal control) primers 466 bp. platinum Taq polymerase. In nucleic acid extraction and purification, colony material from pure cultures is suspended in 100µL molecular grade water. DNA has been isolated from bacterial suspensions of approximately 10^8 cfu/ml. PCR cycling conditions were 2 min at 94°C, 40 cycles of 30 s at 95°C, 30 s at 64°C, 30 s at 72°C, 10 min at 72°C, and cooling at 20°C. The comparison of genomic DNA between isolates and type strains by means of rep-PCR fingerprinting can be a useful identification test Rep-PCR tests for identification of bacteria (EPPO, 2010).

DNA barcoding methods

Molecular characterization using multilocus sequence analysis

The distribution of the bacterial spot pathogens to the four Xanthomonas species previously required complex DNA:DNA homology analysis, which is beyond the capabilities of most diagnostic laboratories. Comparisons of commercially sequenced PCR products amplified from selected housekeeping gene loci, on the other hand, now provide equivalent resolution at a significantly reduced cost and effort. Single gene sequencing approaches are used by Parkinson *et al.* (2009) and multilocus sequencing approaches used by Young *et al*. (2008) and Hamza *et al.* (2010), for example, have been shown to adequately differentiate strains of *X. gardneri, X. vesicatoria, X. euvesicatoria,* and *X. perforans*. One set of PCR primers was designed to amplify genes required for a rhs family gene homologous to rhsA, cell envelope biogenesis, and outer membrane biogenesis.

In a PCR reaction using the X.pv.v primer set; only a 517bp PCR product was produced. A highly specific, sensitive, and rapid PCR assay for detecting Xanthomonas. The method can be used as a reliable diagnostic tool for detecting X.pv.v in pepper or tomato (Park *et al.,* 2009). PCR primer sequences for detecting bacterial spots on tomatoes have been distributed (Park *et al.,* 2009).A numbers of xanthomonads have had specific primers and DNA probes developed for identification and detection (Park *et al.,* 2006). Species-specific primers were created from scratch to allow the identification of a single or multiple, but not all, species of the Xanthomonas complex associated with bacterial spot (Moretti *et al.,* 2009). Furthermore, the PCR must be followed by restriction analysis using a set of enzymes (CfoI, HaeIII, or TaqI), which is more time consuming than a specificPCR.Plant diseases caused by Xanthomonas spp. have been successfully diagnosed using species-specific primers (Park etal. 2006). Leite Jr *et al.* (1995) used RST 65/69 primers to amplify DNA from four species (groups A, B, C, and D) associated with bacterial spot. A multiplex PCR protocol for the simultaneous detection of three seedborne tomato phytobacteria, including *X. axonopodis* pv. *vesicatoria* now also known as *X. euvesicatoria* (Young *et al*., 2008).

Multiplex PCR detection has become increasingly common in diagnostic processes (Hamza *et al.,* 2010). Using this method, it was possible to identify dissimilar species that may be related epiphytically or living in host tissues at the same time. The main goal of the multiplex method is to detect and identify one or more of the bacterial spot's causal agents in a single step. The multiplex protocol appears to be still appropriate for identifying a single species causing a

bacterial spot in a sample. Furthermore, because the crude extract of leaves may contain PCR inhibitors, direct detection from symptomatic leaves can be easily accomplished with a 1:10 dilution of the plant extract for both conventional and multiplex PCR. However, the presented multiplex PCR needs to be further optimised for improved sensitivity results. According to Mahuku (2004), DNA was extracted and quantified on agarose gels by visual comparison with the marker DNA High Mass Ladder. DNA samples were diluted to about 50 ng/l and stored at 20°C until use. Thus, Bio-multiplex PCR could be used to detect viable bacterial cells while also identifying all bacterial spot species associated with asymptomatic greenhouse grown transplants and seed.It is still worth noting that such samples are likely to have a low concentration of bacterial cells. Furthermore, depending on their origin, previously unknown bacterial spot xanthomonads, for example, could be involved in the sample infection (Pernezny and Collins, 1997). The use of PCR with a hot start or nested PCR (Hamza *et al.,* 2010) has been shown to improve the sensitivity and effectiveness of multiplex PCR. These procedures have been effective in lowering PCR inhibitor factors such as the formation of primer dimers prior to the start of thermocycling (4-25°C). Changes in reagent concentrations, such as primers, magnesium, and Taq DNA polymerase, must be optimised because they have a significant impact on the quality and reproducibility of the results.Sensitivity of specific primers in conventional PCR was determined for *X. vesicatoria* and the sensitivity of each primer pair using both purified DNA and bacterial suspensions. Bs-XvF/Bs-XvR primer pairs for *X. Vesicatoria* were less sensitive, amplifying target DNA in suspensions the equivalent of 100 bacterial cells per reaction.

Multilocus sequence analysis (MLSA) is currently being used to classify Xanthomonas species. MLSA suggests a flexible, relatively simple, and efficient method similar to conventional DNA-DNA hybridization and 16S rRNA sequencing methods (Young *et al.,* 2008). Based on MLSA, a DNA-based method for studying relationships between species in the genus Xanthomonas was developed in which four gene sequences were used to identify allelic mismatches at different xanthomonad loci: the chaperone protein dnaK (dnaK), B-dependent receptor (fyuA), DNA gyrase subunit B (gyrB), and RNA polymerase sigma factor rpo (Young *et al.,* 2008). Several systems for characterising xanthomonads based on their housekeeping genes have been proposed (Almeida *et al.,* 2009). Almeida *et al.* (2010) suggested using six housekeeping genes (fusA, gapA, gltA, gyrB, lacF, and lepA) to distinguish between Xanthomonas species and also to characterise strains below the species level by looking at variation within sequences. Multiple recombination events between Xanthomonas euvesicatoria and *X. perforans* strains were observed using these genes for strain comparison (Timilsina *et al.,* 2015). Total DNA was extracted from the bacterial strains using the CTAB-NaCl extraction method (Ausubel *et al.,* 1994) and resuspended in TE buffer.

HrpB genes were sequenced in strains, and six housekeeping genes were sequenced in strains chosen for their non-redundant novel sequences as seen in hrpB genes and their phenotypic responses. Loci were amplified using degenerate primers specific to them (Almeida *et al*., 2010). A single pure colony was suspended in 100 L milliQ water, incubated at 95 °C for 7 minutes, and then used for species specific and MLSA associated PCRs. All isolates containing type strains were tested using PCR procedures developed to differentiate *X. euvesicatoria, X. gardneri*, *X. perforans*, and X. vesicatoria (Koenraadt *et al.,* 2009). As previously described, MLSAPCR primers targeting the chaperone protein dnaK (dnaK), elongation factor P (efp), ATP synthase subunit beta (atpD), and DNA gyrase subunit B (gyrB) genes were used (Hamza *et al.,* 2012). The MLSA primers targeting all four genes had their annealing temperatures optimised. The MLSA results suggested that *X. perforans* and *X. euvesicatoria* have a close phylogenetic relationship. Using MLSA, Young *et al.* (2008) proposed that the species *X. perforans* and *X. euvesicatoria* be treated as synonyms. MLSA in conjunction with rep-PCR appears to be of interest for epidemiological studies (Feng *et al.,* 2009). Potnis *et al.* (2011) compared the complete genome sequences of all four tomato bacterial spot species and determined their separation into four entities. DNA sequencing is a powerful method for new species descriptions and reclassification proposals, but it is expensive, requires more specialised equipment, and is not always suitable for monotonous diagnosis.

DNA amplification and restriction endonuclease study

In order to develop a sensitive method for rapid detection of X pv.v we sequenced the PCR products of 10 representative A, 6 B, 20 C, and 3 D group strains (Jones *et al.,* 2000) following amplification of the HrpB region of the *hrp* gene cluster of X.pv.v with RST2/RST3 (840 bp) (Leite Jr *et al.,* 1995) and designed new primers based on conserved sequences within the regions of the four groups. The set of primers designated RST65 (5 GTCGTCGTTACGGCAAGGTGGTCG3) and RST69 (5 TCGCCCAGCGTCATCAGGCCATC 3) amplify a fragment of genomic DNA from all tested 'vesicatoria' strains and some other pathogenic Xanthomonas sp. or pathovars. In order to determine the grouping of the 'vesicatoria' strains, the PCR products were digested with restriction enzymes CfoI, TaqI, and HaeIII.

Restriction analysis

Restriction analyses (Leite Jr *et al*., 1995) and repPCR (Pereira *et al.,* 2011) have been used to characterise Xanthomonas strains causing bacterial spot, but these methods frequently require highquality purified DNA and gel electrophoresis to generate highresolution and reproducible fingerprints (Ishii and Sadowsky, 2009). The designated primer set (RST65/69) produced a PCR product from the genomic DNA of all tested'vesicatoria' strains as well as 9 out of 12 pathogenic Xantomonas sp. or pathovars. Saprophytic bacteria and opportunistic xanthomonads yielded no PCR product. The banding patterns obtained by cutting the PCR product of 39'vesicatoria' strains with the restriction enzymes CfoI, TaqI, and HaeIII allowed four groups to be distinguished (A, B, C, D). The same process was used to analyse eight pepper and three tomato strains isolated from different areas and years in Serbia. When the amplified 420 bp product from the pepper strains was digested with HaeIII, it had the same pattern as the illustrative phenotypic group A and C strains, whereas the tomato strain patterns clustered with the B group representative strain.

Management and control of pathogen

So far, no method has been developed that can ensure the complete eradication of pathogens from naturally

diseased seeds without significantly reducing seed germination (Dhanvantari, 1989). AL-Saleh (2011) stated that once the disease is present in the crop, it is difficult to control and causes significant fruit loss in tomatoes when environmental conditions are favourable. Despite the use of phytosanitary certification and quarantine procedures in domestic and international seed trade, which can reduce disease occurrence significantly, severe epidemics are occasionally described (Gitaitis and Walcott, 2007). Chemical pesticides, transgenic resistant hosts, and host resistance, cropping systems, and biological control agents have all been used to combat bacterial spot (Bardin *et al.,* 2015). BLS is primarily controlled through the use of resistant lines, antibiotic application, and copper sprays. Because Xanthomonas strains rely on a limited set of chemicals, copper and antibiotic resistance has emerged quickly (Griffin *et al.,* 2017; Klein-Gordon *et al.,* 2021).

Transplants are the preferred planting material in both conventional and organic systems because they avoid the difficulties associated with direct seeding and allow the crop to establish quickly and uniformly in the field (Boyhan and Kelley, 2014). Seed and transplants are ideally produced in cool, arid areas certified diseasefree to reduce the risk of infested planting material (Langston, 2014), but this strategy is not always successful (Darrasse *et al.,* 2007), especially with a pathogen that can colonise the host and contaminate seed without producing symptoms (Dutta *et al.,* 2014). Sanitation, the use of pathogen free seed, and other cultural practises, as well as the use of tomato cultivars resistant to *X.* pv. *vesicatoria*, have all been used to control the disease (Jones *et al.,* 1991; Bouzar *et al.,*1999). Cultural practises can reduce the likelihood of disease development. Bacterial spot management should include sanitation, crop rotation, and drip, furrow, or trickle irrigation. Xanthomonas only survives 16 days in sandy soil with no viable host material, but several months in host-free loam soil, up to several months on crop residues depending on decomposition rate, and up to twelve months on volunteer tomatoes (Stall *et al*., 2009; Ryan *et al*., 2011).

Select pathogen free seed from a reputable source

In arid or semiarid areas, or during the dry season, seed production is one of the first steps in bacterial spot prevention. One of the most common forms of protection is the use of certified and declared seeds. Though it is often easier said than done, it is critical to obtain the highest quality seed possible. Because the seed extraction process will not reliably remove bacteria from the seed, treating the seed with chlorine bleach to disinfest the seed surface or a hot water seed treatment to disinfest the seed surface and remove bacteria that may be under the seed coat may be necessary. Before sowing, it is critical to disinfect the seeds with Clorox Liquid Bleach (5% sodium hypochlorite), 0.8% acetic acid at 21°C for 24 h or 5% hydrochloride. Avoid using seed produced by overhead irrigation, as well as performing operations around the plants while the foliage is wet. Crop rotations, certified diseaseas seeds and seedlings, proper disposal of infected plant debris, multiple treatments of copper bactericides, and biological control are used to control BLS in organic tomato production (Potnis *et al.,* 2015).

Sanitation during transplant production

Sanitation eliminates initial inoculum sources that could lead to disease the following season.

Sanitation is an essential part of transplant production. Given that the pathogen can survive for months on crop residues, an oneyear crop rotation with nonsolanaceous crops is recommended, but it may not be economically feasible (Langston, 2014). The pathogen can survive on the roots of plants in the Solanaceae family. As a result, one of the primary preventative measures is the destruction of weeds from the Solanaceous family and plants that can serve as hosts for the pathogen. Disinfectants used to clean tools and work surfaces, as well as skinsafe disinfectant washes, reduce the risk of transmission by workers and their equipment (Pohronezny *et al.,* 2010).

Sanitary measures, such as keeping the field weed-free, have been proposed and have been found to correlate positively with low BLS occurrence and severity (Shenge *et al.,* 2010). A survey conducted in the country revealed that the BLS disease of tomato is widespread, affecting all cultivars grown (Shenge *et al.,* 2010). Among these are the following: removing all plant material and weeds from the greenhouse between crops. If reusing trays, use sterile potting mix and new trays, and sanitise them with a disinfectant once all organic matter has been removed. Keep in mind that in the presence of organic matter, disinfectant products will be neutralised and rendered ineffective. Before the growing season begins, disinfect tools and equipment as well as greenhouse surfaces. Avoid mixing seed lots during seeding and in the greenhouse.

Many of the seedlings may be infected but not yet showing symptoms, and some may not show symptoms until they are planted in the field. Reduce leaf wetness during transplant production by timing watering, managing relative humidity, and ventilating. Bottom watering or using low pressure nozzles for watering will reduce potential plant damage and bacterial entry points. Also, do not handle wet plants or allow them to fall on each other when preparing for shipping. Solanaceous weeds may serve as hosts, providing inoculum for the following season, but they may not serve as hosts for the pathogen's long-term survival (Gitaitis *et al.,* 1992). As a result, weed control and the disking or elimination of postharvest debris and volunteers are required. In the absence of crop residue, the bacterial spot pathogen cannot survive in the soil. Between periods, manage any solanaceous weeds or volunteers that may harbour bacterial spot. Remove or destroy diseased tomato plants and crop debris. It is also critical to define maintenance and balanced fertility, because plants that grow in the absence of nutrients are susceptible to pathogen attack (Sikora, 1994). Sanitation in the greenhouse is required for the production of transplants. Disinfect equipment, walls, benches, tools, and wash hands thoroughly. Crop rotation is also required in addition to these measures. After 3 to 4 years, tomatoes may appear on the same surface. Reduce crop stress by encouraging good soil drainage, adequate crop fertility, and proper air circulation. When possible, use drip irrigation. When possible, separate seed lots to reduce potential spread if one seed lot is infested. Separate sequential plantings and begin with the youngest. Once the main fruit crop is established, late-season foliar symptoms and potential fruit spread become less problematic. Tilling down crop residue

soon after harvesting will help crop residue decay and reduce spread to younger successive plantings.

Research and breeding for resistance

Plant breeders are now using bacterial spot resistance genes to create resistant varieties. The goal of this study is to investigate the perceived relationship between pathogen and host, as well as the methods bacteria use to come into contact with the host and cause disease. The genetic variability of Xanthomonas, which causes tomato bacterial spot, is extremely broad. As a result, it is nearly impossible for plant breeders to select a variety that is consistently resistant to a specific species of pathogen. Lin *et al.* (2004) investigated the potential of tomato disease resistance using genetic engineering. They used SAR-related genes in this study to increase tomato resistance to a variety of diseases. The Arabidopsis NPR1 gene was introduced into tomato cultivars for this study. The mentioned diseases did not develop in transgenic lines over the next four years, demonstrating that this method is very effective in disease suppression. Yang and Francis (2005) improved tomato resistance by crossing two elite breeding lines. They crossed the lines Ohio 9811205, which carries the pto gene and is resistant to bacterial spot, and Ohio 9834, which carries the locus Rx3 and is resistant to bacterial spot. For coupling-phase resistance, they used Marker-Assisted Selection (MAS) in the F2 progeny. This study discovered that all 33 families tested positive for bacterial spot resistance. Scott Somodi *et al.* (1989) are monitoring the resistance of tomato varieties to Xcm using various experiments. They followed the level on the varieties Sugar and Havaii 7998.The sugar variety was more sensitive to the appearance of symptoms on the leaves than the fruits. Havaii 7998 had more developed symptoms on the fruits. They attempted to create a resistant variety by crossing these two varieties. However, statistical data from the next generation revealed that there is no difference between offspring and parents. The most desirable method of disease management is host resistance to bacterial spot. Tomato cultivars that are resistant to tomato and pepper bacterial spot pathogen races have been identified (Bonshtien et al., 2005; Yang *et al.,* 2005). However, these cultivars have not been widely adopted (Langston, 2014), possibly because the resistance is not long lasting (Stall *et al.,* 2009; Clarke *et al.,* 2014), and the available resistant cultivars are not resistant to all strains of the pathogen.

Chemical methods

The use of fixed-copper fungicide is the primary chemical control of tomato. This requires the use of copper salts, which are water-insoluble. Copper has been used as a fungicide in agriculture since the early 1800s. The Bordeaux mixture, which was accidentally exposed in 1885 by the French scientist Pierre-Marie Alexis Millardet, was the first copper-based antimicrobial compound (CBAC) used in farming (Gayon and Sauvageau, 1903). Copper is an essential micronutrient for all plants and serves as a cofactor for several enzymes involved in respiration and electron transport proteins (Sommer, 1931). At the same time, Cu acts as a broad-spectrum biocide at higher concentrations due to its interactions with nucleic acids, disruption of enzyme active sites, and interference with enzyme activity (Strayer-Scherer et al., 2019).

Copper ions are known to bind strongly to sulfhydryl groups, which accounts for their biocidal properties. Copper ions can enter plants through their cuticles and cause severe phytotoxicity. The solution is water-insoluble copper salts, which have become the primary chemical group for disease control. Chemical controls, such as copper and streptomycin sprays, have also been employed. Several inorganic Cu preparations have been developed and are being used as biocides to control plant pathogenic bacteria, fungi, oomycetes, and, in some cases, invertebrates and algae (Capinera and Dickens, 2016). CBACs are used for seed treatment to prevent plant pathogen infection of seedlings in addition to direct treatment on plants in the field (Verma *et al.,* 2011).

Itako *et al*. (2015) discovered fixed copper compounds have broad-spectrum bactericidal activity, and foliar sprays are effective in reducing the impact of bacterial spot and other bacterial diseases on tomato. They act as protectants and must come into contact with the pathogens prior to infection. Fixed copper-based products are still the primary tool for managing bacterial spot in tomato in the field. Coppers must be used as soon as possible, usually at the first sign of disease or the appearance of the first true leaves (Sun *et al.,* 2002). When copper compounds are tank-mixed with ethylene bisdithiocarbamate (EBDC) fungicides, they are more effective. The mechanism by which it increases its efficacy is not well understood. Marco and Stall (1983) proposed that it increases the amount of available copper, but Hausbeck *et al.* (2000) disagreed, proposing a different mechanism.Copper can be tank mixed with mancozeb in non-certified organic schemes to boost the bactericidal effect of fixed copper and help manage any copper resistant strains. Actigard 50WG (acibenzolar-S-methyl) has been shown in a number of research-based trials to help reduce the frequency and harshness of bacterial spots when applied early in the season. This activates the plant's own defence system, which produces proteins and other products that allow the plant to defeat pathogens. These defence mechanisms are only activated when a plant detects a pathogen. Priming, or pre-activating the plant's defences, improves the plant's defence response.

Copper is typically applied to dry seedlings prior to transplantation to the production field in conjunction with maneb spray. These chemical components kill bacteria found on the surfaces of the plant. If the disease reoccurs after the transplant, the new protection must be applied every 10 to 14 days. The application of chemical agents is heavily influenced by environmental factors. Conditions that promote disease development reduce the effectiveness of cooper-maneb spray (Graves and Alexander, 2002). Copper xydroxide, copper salts combined with mancozeb, copper salts of oil and rozin acids, and copper penta xydroxide were all used as protection. Currently, the most effective treatment for tomato bacterial spot is a combination of chemical applications. To kill bacteria on the seed's surface, diseased seeds can be treated with sodium hypochlorite, calcium hypochlorite, or trisodium phosphate. Internal seed infection is more difficult to treat, but it is possible with other chemical or heat treatments. Multiple application methods are used to treat infected fields, including spraying a mixture of copper and mancozeb, which prevents further infection and kills a variety of pathogens present at the time of spraying. Finally, some research is being conducted into the foliar application of bacteriophages to control the disease, but this approach

has encountered some resistance and is not currently a widely used method.

Copper resistance has been discovered in a variety of plant pathogens (Behlau *et al.,* 2011). In most cases, copper resistance has been linked to plasmids, and to a lesser extent, copper resistance genes have been linked to chromosomes (Behlau *et al.,* 2017). Copper-tolerant strains of *X. euvesicatoria* were found to be sensitive to copper bactericides when mixed with ethylene-bis-dithiocarbamates (Marco and Stall, 1983); however, copper-mancozeb has not been consistently effective in controlling bacterial spot or increasing yield during optimal disease conditions (Obradovic *et al.,* 2004).

The risk of copper phytotoxicity (Ouariti *et al*., 1997) and the development of copper tolerance in some strains of the pathogen (Jones *et al*., 1991) add to the difficulty of using this chemical tool. Copper resistance genes are found on plasmids, which can be passed on to other bacteria (Behlau *et al*., 2013; Bender *et al*., 1990). The use of EBDCs has raised public health concerns (Yang *et al*., 2005), and they are no longer permitted in some states for use on processing tomatoes (Hausbeck *et al*., 2000).

EBDC compounds are not allowed in organic production. The emergence of copper resistance in strains of Xanthomonas was found to be associated with mobile elements such as plasmids that thus represent a significant risk of rapid and widespread propagation within the bacterial populations (Richard *et al.,* 2017). The efficiency of copper-based bactericides against BLS is further reduced when weather conditions are conducive for the development and spread of this disease, due to the novel xanthomonads virulence factors, which enable them to infect tomato over a wide range of temperatures (Potnis *et al.,* 2015). Another strategy to overcome resistance in Xanthomonas spp., i.e., copper bactericides combined with biofungicides was found to result in reasonable success against BLS (Abbasi and Weselowski, 2015). Hence, BLS management in organic tomato production is a major task, and conventional disease control measures in organic tomato production need to be combined or substituted with more active natural compounds to circumvent bacterial resistance to bactericides. Management of BLS is limited to foliar applications of copper based compounds. However, presence of strains of BLS pathogens with a high degree of tolerance to copper (Shenge *et al.,* 2007) and the considerable number of *Xanthomonas* species and races causing BLS symptoms in tomato and pepper have made the control of the disease tough (Jones *et al.,* 2004).

The main disadvantages of copper ions are that they do not have the possibility of degradation in soil and high phytotoxicity on the plants. Copper materials are usually results in a reduction in sensitivity of cooper among X.pv.v strains and environmental impact. Copper based bactericides are that they have adverse effects on both human and animal health. In current years the demand for chemical free products by consumers has augmented and this has resulted in the restricted use of chemicals (Buttimer *et al.,* 2017). Copper ions are not degraded in soil and can accumulate to high levels at locations with a history of intensive copper application (Koller, 1998). Drawbacks of chemical applications such as potential chemical residues on fruit, cost and development of resistant bacterial strains have been reported (Ritchie and Dittapongitch, 1991).

The main disadvantages of copper ions are that they do not degrade in soil and have a high phytotoxicity on plants. Copper materials typically result in a decrease in sensitivity of copper among X.pv.V strains and an increase in environmental impact. Additionally, the problem with copper-based bactericides is that they are harmful to both human and animal health. Consumer demand for chemical-free products has increased in recent years, resulting in the restricted use of chemicals (Buttimer *et al.,* 2017). Copper ions are not degraded in soil and can accumulate to high levels in areas where there has been extensive copper application (Koller, 1998). Chemical applications have drawbacks such as potential chemical residues on fruit, cost, and the development of resistant bacteria.

Antibiotics have been tried for bacterial spot management but are not presently in common usage. Streptomycin is the most common antibiotic used in plant production, but oxytetracycline and kasugamycin have also been studied in greenhouse and field applications (Sun *et al.,* 2002; Vallad *et al.,* 2010). Antibiotic resistance develops relatively quickly in Xanthomonas and antibiotics may have phytotoxic effects (Mc Manus *et al.,* 2002). Antibiotic use is not advisable in the field except for high value crops because of the high cost and the short time for which the antibiotic is effective (Stockwell and Duffy, 2012). Concerns have been raised about the spread of antibiotic resistance among clinically important bacteria resulting from use of antibiotics in agriculture (Stockwell and Duffy, 2012). Antibiotics have not been recommended for bacterial spot management for over forty years (Stall *et al.,* 2009).

Recently, another chemical control methods have been examined in which chemicals are applied that trigger plant defense responses. Systemic acquired resistance (SAR) is a biochemical state of the plant in which the plant improves resistance to a pathogen by earlier infection by that pathogen or a different pathogen (Sticher *et al.,* 1997). SAR is a mechanism which represents resistance of plant parts that have not been exposed to the pathogen attack. Chemical products that cause SAR in plant are called plant activators. Resistance in plants can be induced by virulent, a-virulent, non-pathogenic microbes and with chemicals such as salicylic acid or 2,6-dichloro-isonicotinic a benzo- (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH). Activation of SAR-system in plants depends on the time period necessary for the buildup of pathogenesis related proteins and passing salicylic acid throughout the plant (Vallad Goodman, 2004).

The most common plant activators that are nowadays used are acibenzolar-Smethyl (ASM), known as Actigard and harpin (Momol *et al.,* 2002). Numerous materials that specifically induce SAR, such as acibenzolar-S-methyl (ASM) and harpin (Messenger, Eden Bioscience, WA) have been examined. ASM is a synthetic plant activator revealed to induce systemic acquired resistance that is as effective as copper- EBDC sprays in controlling bacterial spot on both peppers and tomatoes (Huang *et al.,* 2012). Overall, 21 of 25 studies described effective control of Xanthomonas spp. with ASM applied alone and overall presentation was either better or equivalent to a copper/ EBDC program (Buonaurio *et al.,* 2002). Efficacy is observed whether ASM is applied before

(Huang *et al.,* 2012) or after (Cavalcanti *et al.,* 2007) the plants are inoculated with the pathogen. Host defense mechanisms against bacterial spot are also induced by foliar applications of chitosan (Coqueiro *et al.,* 2011).

ASM was occasionally reported to have negative crop growth and yield effects; however, some trial sites within the studies were not affected (Pontes *et al.,* 2016; Louws *et al.,* 2001; Romero *et al.,* 2001). Negative crop effects are reported to be mitigated by incorporating ASM applications into a fortnightly program alternating with a copper-based bactericide or other products (Roberts *et al.,* 2008). As well as using a maximum of 7to 8 applications of ASM, followed by copper-based products later in the season (Pontes *et al.,* 2016). The desire for clean seed has led to decades of research on seed treatments (Mbega *et al.,* 2012a, 2012b). Seed treatments may be chemical or physical, such as the use of heat. Chemical treatments, such as with hypochlorite (in chlorine bleach) or inorganic and organic acids, sanitize the seed surface while hot water treatments can also reduce bacterial populations within the seed. Hot water seed treatments are usually effective especially if the infestation in the seed lot is relatively low (Mtui *et al.,* 2010).

Inorganic and organic acids have also shown effectiveness as seed treatments. The most commonly used inorganic acid is hydrochloric (Hopkins *et al.,* 2003). Acetic acid (van der Wolf *et al.,* 2008) and peracetic (peroxyacetic) acid (Hopkins *et al.,* 2003) are the most commonly used organic acids but lactic acid and ascorbic acid also showed promising results (van der Wolf *et al.,* 2008). Chlorine compounds are widely used disinfectants, especially sodium hypochlorite (NaOCl) which is the active ingredient in chlorine bleach. Sodium (or calcium) hypochlorite is also frequently used in laboratory investigations to sanitize seed or seedling surfaces in preparation for experimental work. The recommendations for the use of hypochlorite in seed treatments can vary from 0.5% to 20% in a water mixture and the duration of soaking from 2 to 40 min (Kordali *et al.,* 2008). In all cases, the seeds were thoroughly rinsed in water after soaking in order to minimize any negative impact on seed vitality (Khah and Passam, 1992). Hypochlorite concentrations equivalent to less than 1% bleach can kill some bacterial spores within 5 to 10 minutes (Rutala *et al.,* 2008). Organic matter (Ivancev-Tumbas *et al.,* 1999), bacterial biofilms (Jaglic *et al.,* 2012; Wirtanen and Salo, 2003) and possibly the xanthans produced by Xanthomonas species (Maude, 1996) can quickly neutralize chlorine compounds and reduce the efficacy of disinfectants. In heavily infested seeds, especially where pathogens may be clumped on the surface, the biofilm may protect some pathogens from exposure to the treatment. Therefore, hypochlorite concentrations need to be high enough to compensate for these challenges but without damaging the seed (Khah and Passam, 1992).

Biological control

Presently, the management of bacterial spot disease depends mostly upon the use of bactericides, such as copper compounds. In overall, the control of various pathogens of Xanthomonas is carried out by use of less susceptible species or varieties, the use of windbreaks, disinfestation of equipment and tools, the addition of copper based chemicals and the eradication of polluted plants (Peitl *et al.,* 2017). But, the increasing public concerns about food safety and environmental hazards have resulted in a decrease of dependence on chemical bactericides and antibiotics in developed and some developing countries. The use of resistant host varieties and application of chemical compounds as cupric bactericides are conventional control methods for bacterial spot (Peitl *et al.,* 2017).

The majority of bacterial spot disease management is currently based on the use of bactericides such as copper compounds. Overall, Xanthomonas pathogens are controlled by using less susceptible species or varieties, windbreaks, deinfestation of equipment and tools, the addition of copper-based chemicals, and the eradication of polluted plants (Peitl *et al.,* 2017). However, owing to the growing public concern about food safety and environmental hazards, developed and some developing countries have reduced their reliance on chemical bactericides and antibiotics. Hence biological control for infestations is also being explored, among those are the Traditional bacterial spot control methods that include the use of resistant host varieties and the application of chemical compounds as cupric bactericides (Peitl *et al.,* 2017).

However, these approaches have showed to be ineffective for the control of bacterial spot (Pernezny *et al*. 2012). Thus, numerous studies involving biological control for bacterial spot of the tomato have been established (Moss, 2007). The bacterial pathogen *X.pv.v* is prevalent in this region for over two decades in spite of much application of the pesticides on the crop to manage this bacterial disease (Borkar and Yumlembam, 2016). This may be due to the development of pesticide resistance in the bacterium, thereby making the pesticide/agrochemicals ineffective in its management. The rate of formation of pesticide-resistant mutant against the pesticide also varies with the pesticide and the strain of the pathogen (Anderson, 2006; Araujo *et al.,* 2012). However, these measures have been incompetent, due to the buildup of chemical substantial in the environment, susceptibility of supposedly resistant varieties and planted area loss (Dewdney and Graham, 2017).

These methods, however, have proven ineffective for bacterial spot control (Pernezny *et al*., 2012). As a result, numerous biological control studies for tomato bacterial spot have been established (Moss, 2007). Despite extensive crop pesticide use to manage this bacterial disease, the bacterial pathogen *X.pv.v* has been present in this region for more than two decades (Borkar and Yumlembam, 2016). This could be because the bacterium develops pesticide resistance, rendering pesticides or agrochemicals ineffective for controlling it. The rate of formation of pesticide-resistant mutants against the pesticide also varies depending on the pesticide and pathogen strain (Anderson 2006; Araujo *et al.,* 2012). These measures, however, have been ineffective.

The biological control of plant diseases is a promising an eco-friendly alternative to chemical pesticides, for preserving plant health and promoting crop yield. It is being studied widely on numerous plant diseases (Areas *et al.,* 2015). The microbes based products are used in plantations and greenhouses to decrease diseases in many cereals, vegetables, fruits, flowers and ornamental plants caused by foliar or post-harvest pathogens (Cawoy *et al.,* 2011). There are four main biological mechanisms that contribute to disease suppression, competition for space and nutrients, production of specific agents that invoke antibiosis,

parasitism and induced resistance against plant pathogens (De Clercq *et al.,* 2004). Biological control organisms such as antagonistic bacteria and fungi as well as plant growth promoting rhizosphere (PGPR) bacteria have been used as seed treatments and in foliar applications (Fontenelle *et al*., 2011). PGPR is bacteria which inhabits plant roots and soil around the plants. In this way bacteria enable normal growth of bacteria and suppression of pathogen. Furthermore, because they exhibit generally different modes of action from conventional pesticides, they may help to suppress resistant pathogens (Bhattacharyya *et al.,* 2016). PGPR have been widely tried for suppressing disease as a result of induction of plant defense responses in the plant (Obradovic *et al.,* 2005). This PGPR is used to inoculate the seed before sowing. After seeding the bacteria develops in the roots and in this way bacterium occupies a living space for other bacteria and restricts access to nutrients. PGPR bacteria can also produce substances that are toxic to pathogens like HCN (Kamilova and Lugtenberg, 2009).The Bacillus, Pseudomonas, Trichoderma and *Crinipellis perniciosa* have been tested against BLS under field conditions and reported to be effective (Udayashankar *et al.,* 2011). Biological control using microorganisms and their products has been shown to be efficient in the control of the Xanthomonas species (Príncipe *et al.,* 2018). Thus, this review is a report concerning the recent advances in the search for microorganisms for the biocontrol of several Xanthomonas that are important for the world economy.

As previously described in this study, the bacteria of the genus *Bacillus* have presented potential as a biocontrol agent against some species of Xanthomonas Certain studies also show that the genus *Bacillus* can also be a substitute to combat the BLS of tomato and pepper, caused by *X. vesicatoria* (Lanna Filho *et al.,* 2013). In current research, plant growth-promoting bacteria (PGPB) have been recommended as a potential alternative to disease management method since PGPB are known for their growth promotion and disease decrease in crops (Yim *et al.,* 2014; Ferraz *et al.,* 2015). Amongst several PGPB, *Bacillus* sp. (*Bacillus amyloliquefaciens, B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides, B. sphaericus* etc.) is considered as likely candidate due to its antagonistic activity against fungi and bacteria, high spore production ability, antibiotics, lytic enzymes and resistance to adverse environments (Lanna *et al.,* 2013). The genus Bacillus are considered as potential biological control agents due to their broad spectrum antagonistic activity against fungi and bacteria, high spore production capacity and resistance to unfavourable conditions (Ferraz *et al.,* 2015). More recently, the protein fractions from *Bacillus amyloliquefaciens* and *Bacillus pumilus*, isolated from tomato stem, were capable of promote the induction of tomato plants resistance against *X. vesicatoria* (Lanna Filho *et al.,* 2013). *Bacillus pumilus* and *Paenibacillus macerans* epiphytic bacteria inhibited the *X. vesicatoria* growth in the antibiosis test and protected the tomato plants (Lanna Filho *et al.,* 2010). Fluorescent *Pseudomonas* spp. are considered one of the most significant biocontrol agents, they have been described to play a role in controlling many plant diseases caused by bacteria, fungi and nematodes (Kavitha and Umesha, 2007). Some studies have also established the action of these bacteria against *X. vesicatoria* (Príncipe *et al*., 2018). Tailocins produced by *P. fluorescens* SF4c, were efficient in controlling the bacterial spot disease in tomatoes on the plant and it reduced the symptoms on tomato fruit even when applied 12 h after infection (Príncipe *et al.,* 2018).

In the antibiosis test, *Bacillus pumilus* and *Paenibacillus macerans* epiphytic bacteria inhibited *X. vesicatoria* growth and protected tomato plants (Lanna Filho *et al.,* 2010). Fluorescent *Pseudomonas* spp. are regarded as one of the most important biocontrol agents, having been shown to play a role in the control of many plant diseases caused by bacteria, fungi, and nematodes (Kavitha and Umesha, 2007). Some research has also shown that these bacteria are effective against *X. vesicatoria* (Prncipe *et al*., 2018). Tailocins produced by *P. fluorescens* SF4c were effective in controlling bacterial spot disease in tomatoes on the plant and reduced symptoms on tomato fruit even 12 hours after infection (Prncipe *et al.,* 2018). Fernandez *et al*. (2017) recently reported that tailocins from Recently, Fernandez *et al*. (2017) described that tailocins from *P. fluorescens* SF4c have *in vitro* antimicrobial activity against *X. vesicatoria* X.pv.V Bv5-4a- synonym of *X. axonopodis* pv *vesicatoria* X.pv.V Bv5-4a. Under greenhouse experiments that mixture of two individual PGPR strains (*B. velezensis* AP197 + AP298) and (*Bacillus altitudinis* AP69 + *B. velezensis* AP199) shows well biocontrol against foliar bacterial pathogen (*X. axonopodis* pv. *vesicatoria*) than these strains individually (Liu *et al.,* 2018). The *B. amyloliquefaciens*, *B. subtilis*, *B. pumilus* and challenged with *X. campestris* pv. *campestris* (Wulff *et al.,* 2002). Also, Bacillus strains isolated from pepper controlled the bacterial spot (*X. axonopodis* pv. *vesicatoria*) disease of tomato plants (Mirik *et al.,* 2008).

El-Hendawy *et al*. (2005) described that *Rahnella aquatilis* inoculated tomato seedlings had improved disease resistance against infected by *X.pv.v*. Before the pathogens attack, the seedlings are treated with several strains of *Rahnella aquatilis,* application was conducted on leaves, roots, soil and seeds. Seedlings showed good outcomes in suppression of the disease and reduction of deleterious effect or stress. The most effective method in the application of *Rahnella aquatilis* is foliar treatment of plants, because it has a direct effect on pathogens. The yeast *Pseudozyma churashimaensis* strain RGJ1 isolated from pepper leaves conferred important protection against *X. vesicatoria* in planta and the molecular analysis of the induced resistance marker genes indicated that strain RGJ1 elicited plant defense priming (Lee *et al*. 2017). Filho *et al*. (2010) investigated the possibility of using epiphytic bacteria for biocontrol of bacterial spot (*X. vesicatoria*) by two methods (*in vitro* and *in vivo*). In this research they used two *epiphytic bacteria Peanibacillus macerans* and *Bacillus pumilus*. In the antagonistic activity and antibiosis test they proved that epiphytic bacteria reduced the severity of bacterial spot (*X. vesicatoria*) and reduced phytopathogenic bacterial cells by 70 % of (Filho *et al.,* 2010). One of the sustainable agricultural control approaches against pathogen attacks is an activation of the plant's defence system depicted by induction of systemic resistance through PGPB (Bardin *et al.,* 2015). Defense related enzymes have a broad action spectrum and play an important role in plant pathogen interactions (Bolouri Moghaddam *et al.,* 2015). The activities of defence related enzymes, containing SOD, CAT, POX, and PPO, considerably improved after treated by *B. subtilis* CBR05.

Foliar treatment of tomato plants with protein fractions of endophytic *Bacillus* strains induced the level of POD and PPO, resulting in a decrease in bacterial spot in tomato (Lanna-Filho *et al.,* 2013). Despite the induction of antioxidant enzymes, tomato plants with *X. vesicatoria* also elicited PR proteins like β 1, 3-glucanase, POX, chitinase and proteinase inhibitor. Cavalcanti *et al.* (2006) also described that protective effect and lesion reduction in *X. vesicatoria* infected tomato plants were associated particularly with PPO, POX and to a lesser extent, chitinase activity. Another method consisted of using bacteriocin producing strains that are inhibitory to pathogenic strains of a closely related organism (Hert *et al.,* 2009). These proteinaceous compounds are capable of killing bacteria phylogenetically close to the producer strain (Ahmad *et al.,* 2017). The application of these antibacterials for the biocontrol of phytopathogens, though, has thus far been incomplete, mainly owing to the paucity of research on those compounds' mechanism of action, rather than their intrinsic potential (Grinter *et al.,* 2012). The feasibility of utilising epiphytic bacteria for the biocontrol of bacterial spot (X. vesicatoria) was investigated by Filho *et al*. (2010) using two approaches (in vitro and in vivo). They used two epiphytic bacteria, *Peanibacillus macerans* and *Bacillus pumilus*, in this study. They demonstrated that epiphytic bacteria reduced the severity of bacterial spot (*X. vesicatoria*) and phytopathogenic bacterial cells by 70% in antagonistic activity and antibiosis tests (Filho *et al.,* 2010). One of the long-term agricultural control strategies against pathogens is to activate the plant's defence system by inducing systemic resistance with PGPB (Bardin *et al.,* 2015). Defense-related enzymes have a broad action spectrum and are crucial in plant pathogen interactions (Bolouri Moghaddam *et al.,* 2016). Lactic acid bacteria have also shown action in combating the bacterial blotch of tomatoes. The efficacy of lactic acid bacteria (LAB) isolated from fresh fruits and vegetables as biocontrol agents against *X. vesicatoria* (Trias *et al.,* 2008). The antagonistic activity of LAB strains was tested *in vitro* and the *X. vesicatoria* was efficiently inhibited by *Lactobacillus plantarum* strain TC110 and *Leuconostoc mesenteroides* XM360 respectively isolated from tomatoes and custard apples. Shrestha *et al*. (2014) reported that three LAB strains (KLF01, KLC02 and KPD03) displayed an inhibitory effect against *X. vesicatoria in vitro* and they have significantly reduced the harshness of foliar disease in pepper in greenhouse conditions and in the field. *Trichoderma* spp. has potential activity against numerous phytopathogens and some of its biological control mechanisms are well known (Harman *et al.,* 2004).

Saksirirat *et al*. (2009) displayed that *Trichoderma* spp. also have efficacy in induced acquired systemic disease resistance in tomatoes, including the BLS disease caused by *X. vesicatoria*.. Naturally occurring avirulent strains of *X. campestris* have been evaluated for control of a few *X. campestris* pathogens: for example, *X. campestris* pv. *malvacearum* was evaluated as a possible biological control agent of black rot of cabbage, caused by X.pv.V (Jetiyanon, 1994) and *X. campestris* pv. *raphani* has been used in an attempt to control X.pv.v in tomato (Sahin and Miller, 1997). Near-isogenic, non-pathogenic mutants of a bacterial plant pathogen for the purpose of biological control can be generated through the deletion of genes involved in pathogenicity/virulence (Lindemann, 1985); specifically through the mutation of one or more hypersensitive response and pathogenicity (hrp) genes (Wilson *et al.,* 1998). Such hrp mutants have been evaluated as potential biological control agents of the pathogenic parent in several pathosystems. Substantial progress has been made in understanding hrp gene expression and regulation in X.pv.v (Buttner and Bonas, 2002). Moss *et al.* (2007) have used the *hrp* mutants of X.pv.v 75-3 with mutations in either regulatory genes, hrpG or hrpX, or in structural genes, *hrpX,* hrpE1, hrpF, were evaluated for ability to reduce bacterial spot severity when applied in advance of the wild-type parental pathogen strain under both greenhouse and field conditions. The highest effect in suppressing of disease was demonstrated by the *hprG* mutant, with 53% of disease reduction. After it, follows the *hprX* mutant with 40%, *hprF* with 30% and lastly, *hrpE1* with 21%. In the open field the testing was conducted at three locations. But in field condition, only 75-3S *hrpG* and 75-3 *hrpF* mutants demonstrated significant results in disease suppression. However, on all three locations only the 75-3S *hrpG* showed a high degree of bacteria reduction (76 %). Antibacterial compounds of plant origin have been broadly applied in the cosmetic and food industry and in medicine (Pradhanang *et al.,* 2003). In farming, pesticides of plant origin have been used for controlling fungal and bacterial infections in a number of crops (Mohana and Raveesha, 2006). Compost mixtures and their water extracts have been investigated for bacterial spot management. Used as soil amendments, foliar sprays and seed treatments (Reddy *et al*., 2012), compost and compost extracts are generally effective against bacterial spot only when disease pressure is high. Numerous studies have shown that composts from heterogeneous sources can suppress different plant diseases (Suárez-Estrella *et al.,* 2012), resulting from the activity of abiotic and biotic factors (Joshi *et al.,* 2009). The suppressive capacity of composts is highly variable, even when similar composted materials and application rates are used (Bonanomi *et al.,* 2007) and can be affected by compost ageing (Litterick *et al.,* 2004).

The Use of Bacteriophages for control diseases

One of the methods of biological protection of tomato is the use of bacteriophages; Phages have been used as biological control agents (Jones *et al.,* 2007; Nakayinga *et al.,* 2021; de Sousa et al., 2023). Phages are viruses that infect bacteria. Agriphage is one of the products that are often used to protect the plant and they contain bacteriophages (Momol *et al.,* 2002). Phages are viruses that infect and replicate in bacteria. Phage replication cycles include temperate and lytic pathways with the lytic pathway being the easier and more important pathway for employment in phage biocontrol (Nga *et al.,* 2021. In the lytic pathway the phages bind to the surface of bacteria after which they inject their DNA and replicate inside the cell. This results in the production of phage progeny that lyse and kill the bacteria (Altamirano and Barr, 2019).

Mallman and Hemstreet (1924) isolated the cabbage-rot organism from rot infected cabbage tissue and determined that liquid filtrate from the decomposed cabbage inhibited the growth of *X. campestris* in *in vitro*. As research on bacteriophages progressed, their presence in other plant tissue was determined. Bacteriophages offer a substitute to conventional management approaches for controlling plant diseases affected by bacterial pathogens (Fujiwara *et al.,* 2011; Murugaiyan *et al.,* 2011). Out of 174 biopesticides ingredients registered with the United States Environmental Protection Agency (USEPA, 2016), only one

(bacteriophages) is registered as a standard control against BLS. These viruses are very specific to the bacterial species and when they come in contact, the virus injects its genetic material into the bacterial cell where it replicates and eventually causes the bacterial cell to lysis or break open. The phases of phage infection, from adsorption to the burst of the host cell, and physiologic background were characterized.

Later it became clear that two types of bacteriophages exist: lytic phages that always infect from the outside with their infection resulting in the destructive burst of the host cell and release of phage progeny, and temperate phages that can insert their DNA into the host's DNA and stay in association with the host cell in an inactive form, as a prophage, without destroying it. Bacteriophages are an active biocontrol agent for the management of bacterial spot on tomato (Obradovic *et al.,* 2004). Phages have been identified that are specific to *X. euvesicatoria* but have no effect on other bacterial spot pathogens (Gašic *et al.,* 2011). Phage 31, family Autographiviridae, had the broadest spectrum and lysed 12 out of 12 *Xanthomonas axonopodis* pv. *allii* strains, a trait that may contribute to its biological efficacy (Nakayinga et al., 2021).

The effectiveness of phages is limited by their sensitivity to UV light (Silverman *et al*., 2013), high temperatures, pH changes and other environmental conditions, and they require free moisture in which to diffuse to their host cells. Recently, protective formulations were developed to increase longevity of phages on plant surfaces in the field (Balogh *et al.,* 2002).

Bacteriophages degrade relatively quickly necessitating frequent spray applications (Jones *et al.,* 2007). Phage preparations are more effective when a mixture of bacteriophages is used and protective compounds are added to extend the period for which they are active on leaf surfaces (Obradovic *et al.,* 2004). Recently, research on bacteriophages has focused on improving the formulation in order to increase survival of the bacteriophage on the leaf surface (Balogh *et al.,* 2002). Iriarte *et al*. (2007) observed that the efficacy of bacteriophage formulations, against *X. campestris pv. vesicatoria* was reduced by desiccation, fluorescent and/or ultraviolet light, temperature, and the presence of copper compounds. Therefore, new phage formulations to improve disease control efficacy were subsequently developed in which the presence of powdered skim milk plus sucrose eliminated the reduction of phage survival caused by external influences such as desiccation and temperature, among others (Iriarte *et al.,* 2007).

Essential oils

Naturally occurring and biologically active plant products such as essential oils and organic extracts could be a source of alternate classes of natural biopesticides to serve as templates for new and more effective compounds in controlling plant pathogenic microorganisms. Plant-derived essential oils are considered as non-phytotoxic compounds and potentially effective against various pathogenic bacteria (Vasinauskiene *et al.,* 2006). Interest has been produced in the development of safer antibacterial agents to control plant pathogenic bacteria in agriculture which also include essential oils and extracts (Ozturk and Ercisli, 2007). Essential oils from a number of plant species were shown to have antimicrobial activity in previous *in vitro* assays to a range of phytobacteria including xanthomonads (Oliva *et al*., 2015).

Essential oils have been used for centuries for their putative antiseptic properties. They are formed in aromatic plants as volatile, secondary metabolites. Oil extracts have a strong odor, and can have a protective function against herbivory (Bakkali *et al.,* 2008). Essential oils, which are odorous and volatile products of plant secondary metabolism, have wide applications to control pathogenic bacteria (Ozturk and Ercisli, 2007). Essential oils or their components have also been used against microorganisms in seed disinfection (Lo Cantore *et al.,* 2009) and in food preservation and safety (Solomakos *et al*. 2008). Natural materials resultants from medicinal plants have also shown promising results for controlling diseases of diverse crops (Pereira *et al.,* 2011). As to essential oils, the studies aimed at explaining the mechanisms of disease suppression, recommend that the active compounds of the oils act directly on the pathogens or induce host resistance through the making of phytoalexins, increased pathogenesis-related proteins activity, synthesis of structural compounds and biochemical plant defense (Pereira *et al.,* 2011), resulting in disease reduction (Paul and Sharma, 2002). Essential oils from a range of different plant species were previously shown to have efficacy in control of xanthomonads, including those causing BLS. Sage, clove and BioZell™ (based on thyme oil) performed better at controlling a range of plant pathogenic bacteria than lavender and lemon balm. The chemical compounds carvacrol and thymol found in a range of essential oils was shown to be antibacterial to a range of plant pathogens including *X. vesicatoria*. Recently a number of studies have looked at treating seed with essential oil to combat BLS pathogens (Kotan *et al.,* 2014), however, their efficacy is questionable. Kaaya *et al.* (2003) estimated the efficacy of 11 essential oils of plant origin to control BLS xanthomonads of tomato in seed applications and their effect on seed germination and seedling growth of tomato. Foliar applications and seed treatment using thyme oil suspensions have been effective against bacterial spot of tomato (Altundag and Aslim 2011). Mohana and Raveesha (2006) assessed the effectiveness of numerous oils of plant origin against bacterial diseases, containing the BLS pathogen of tomato in *in vitro* assays, revealing antibacterial activity. Mushrooms have been known to have antibacterial properties and are considered one of the most consistent sources for future new compounds (Barseghyan *et al.,* 2015). A study by Kaur *et al*. (2016) on the application *Lentinula edodes* (shiitake) to combat *X. vesicatoria* exhibited that the mushroom extracts suppressed bacterial spot frequency in tomato foliage *in vitro* but was not effective *in vivo*. *Lentinula edodes* an edible, medicinal fungus of the Basidiomycetes family is used as functional food and is well known for its antibacterial properties (Giavasis, 2014). Numerous antibacterial compounds, including lentinan, lenthionine, eritadenine, protocatechuic acid, p-hydroxybenzoic acid and oxalic acid, have been isolated from *L. edodes* fruiting bodies (Reis *et al.,* 2012).

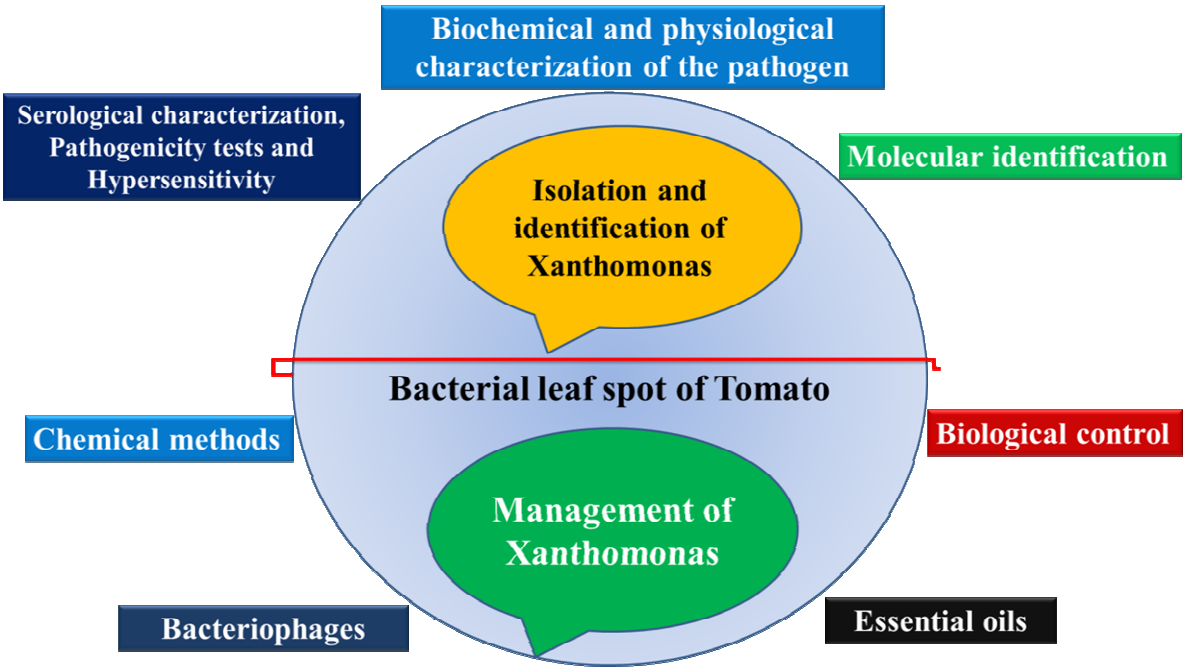

Fig. 3 : Isolation, identification and Management of Xanthomonas

Conclusions

According to the information compiled in this review, the genus Xanthomonas contains a high variety of bacterial plant diseases that affect tomato. The BLS of tomato requires detection systems as well as factors influencing the species specific pathogensis and resistance. The use of functional genomics and proteomics aids in the identification of specific genes and proteins involved in the tomato infection life cycle.

References

Abbasi, P.A., Khabbaz, S.E., Weselowski, B., Zhang, L., 2015. Occurrence of copper-resistant strains and a shift in *Xanthomonas* spp. causing tomato bacterial spot in Ontario. *Can J Microbiol* 61:753–761.

Ahmad, V., Khan, M.S., Jamal, Q.M.S., Alzohairy, M.A., Al Karaawi, M.A., Siddiqui, M.U., 2017. Antimicrobial potential of bacteriocins: in therapy, agriculture and food pre- servation. *Int. J. Antimicrob. Agents* 49, 1–11.

Almeida, N. F., Shuangchun, Y., Magdalen, L., David, J. S., David, J. S., Bradford, C., Haijie, L., Carlos, J. V., et al. (2009). Draft Genome Sequence of *Pseudomonas syringae* pv. tomato, T1 Reveals a Type III Effector Repertoire Significantly Divergent from That of *Pseudomonas syringae* pv. tomato DC3000. *Molecular Plant Microbe Interactions*, 22(1), 52– 62.

Almeida, N. F., Yan, S., Cai, R., Clarke, C. R., Morris, C. E., Schaad,N.W., Schuenzel, E. L., Lacy,G. H., Sun,X., Jones, J. B., Castillo, J. A., Bull, C. T., Leman, S., Guttman, D. S., Setubal, J. C., and Vinatzer, B. A. (2010). PAMDB, A multilocus sequence typing and analysis database and website for plant-associated microbes. *Phytopathology*, 100(208), 215.

AL-Saleh MA. 2011. "Pathogenic Variability among Five Bacterial Isolates of *Xanthomonas campestris* pv. *vesicatoria*, Causing Spot Disease on Tomato and their Response to Salicylic Acid." *Journal of the Saudi Society of Agricultural Sciences* 10(1), 47-51.

Altamirano FLG, Barr JJ. Phage therapy in the postantibiotic era. Clin Microbiol Rev. 2019; 32(2):e00066–18.

Altundag, S., and Aslim, B. 2011. Effect of some endemic plant essential oils on bacterial spot of tomato. *Journal of Plant Pathology* 93(1):37-41.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current protocols in molecular biology. New York: John Wiley and Sons.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. 2008. Biological effects of essential oils – A review. *Food and Chemical Toxicology* 46:446–475.

Balogh, B., Jones, J. B., Momol, M. T., Olson, S. M., Obradovic, A., King, P., and Jackson, L. E. 2003. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Dis.* 87:949-954.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., and Nicot, P. C. (2015). Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Frontiers in Plant Science*, 6, 566.

Barseghyan, G.S., Barazani, A., Wasser, S.P., 2015. Medicinal mushrooms with anti- phytopathogenic and insecticidal properties. In: Petre, P. (Ed.), Mushroom Biotechnology: Developments and Applications. *Academic Press*, pp. 137-154.

Behlau, F., Canteros, B. I., Minsavage, G. V., Jones, J. B., and Graham, J. H. (2011). Molecular characterization of copper resistance genes from *Xanthomonas* citri subsp. citri and *Xanthomonas alfalfae* subsp. *citrumelonis*. *Appl. Environ. Microbiol.* 77, 4089–4096.

Behlau, F., Gochez, A. M., Lugo, A. J., Elibox, W., Minsavage, G. V., Potnis, N., et al. (2017). Characterization of a unique copper resistance gene cluster in *Xanthomonas campestris* pv. *campestris* isolated in Trinidad, West Indies. *Eur. J. Plant Pathol.* 147, 671–681.

Black R, Sweetmore A (1994). Appropriate bacterial identification systems for small plant pathology laboratories overseas incorporating the Biolog method. *Plant Pathol.* 43:438-441.

Bonanomi G., Antignani V., Pane C., Scala F., 2007. Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* 89: 311-324.

Bonshtien, A., Lev, A., Gibly, A., Debbie, P., Avni, A., and Sessa, G. 2005. Molecular properties of the *Xanthomonas* AvrRxv effector and global transcription changes determined by its expression in resistant tomato plants. MPMI 18(4):300-310.

Borkar, S. G., and Yumlembam, R. A. (2016). Bacterial Diseases of Crop Plants. CRC Press.

Borkar, S.G. 2018. Laboratory techniques in plant bacteriology. CRC Press, USA. p.320.

Bouzar H, Jones JB, Stall RE, Hodge NC, Minsavage GV, Benedict AA and Alvarez AM (1994) Physiological, chemical, serological, and pathogenic analyses of a worldwide collection of *Xanthomonas campestris* pv. *vesicatoria* strains. *Phytopathology* 84: 663–671

Bouzar, H., Jones, J.B., Minsavage, G.V., Stall, R.E. and Scott, J.W. 1994. Proteins unique to phenotypically distinct groups of *Xanthomonas campestris* pv. *vesicatoria* revealed by silver staining. *Phytopathology* 84:39-44.

Bouzar, H., Jones, J.B., Stall, R.E., Louws, F.J., Schnei- der, M., Rademaker, J.L.W., de Bruijn, F.J., Jackson, L.E., 1999. Multiphasic analysis of Xanthomonads causing bacterial spot disease on tomato and pepper in the Caribbean and Central America: evidence for common lineages within and between countries. *Phytopathology* 89, 328–335.

Boyhan, G. E., and Kelley, W. T. 2014. Transplant production. Commercial Tomato Production Handbook. UGA Extension. University of Georgia, Athens, GA. Retrieved 28 August 2014 from http://extension. uga.edu/publications/ files/pdf/B%201312_5.PDF

Büttner, D., Bonas, U., 2010. Regulation and secretion of *Xanthomonas* virulence factors. *FEMS Microbiol. Rev*. 34, 107–133.

Capinera JL, Dickens K (2016). Some effects of copper-based fungicides on plant-feeding terrestrial molluscs: a role for repellents in mollusc management. *Crop Prot* 83:76–82.

Cavalcanti, F. R., Resende, M. L. V., Carvalho, C. P. S., Silveira, J. A. G., & Oliveira, J. T. A. (2006). Induced defence responses and protective effects on tomato against Xanthomonas vesicatoria by an aqueous extract from Solanum lycocarpum infected with Crinipellis perniciosa. *Biological Control*, *39*(3), 408-417.

Chandrasekaran, M., & Chun, S. C. (2016). Expression of PR-protein genes and induction of defense-related enzymes by Bacillus subtilis CBR05 in tomato (Solanum lycopersicum) plants challenged with Erwinia carotovora subsp. carotovora. *Bioscience, biotechnology, and biochemistry*, 80(11), 2277-2283.

Chitarra LG, Langerak CJ, Bergervoet JHW, van den Bulk RW. Detection of the plant pathogenic bacterium *Xanthomonas campestris* pv. campestris in seed extracts of Brassica sp. applying fluorescent antibo- dies and flow cytometry. *Cytometry* 2002; 47:118–26.

Chun, W.C., and Alvarez, A.M. 1983. A starch-methionine medium for isolation of *Xanthomonas campestris* pv. *campestris* from plant debris and soil. Plant Disease 67:632-635.

Clarke, C. R., Hayes, B. W., Runde, B. J., Wicker, E., and Vinayzer, B. A. 2014. Eggplant and related species are promising genetic resources to dissect the plant immune response to *Pseudomonas syringae* and *Xanthomonas* eu*vesicatoria* and to identify new resistance determinants. *Molecular Plant Pathology* 15(8):814- 822.

Constantin, E.C., Cleenwerck, I., Maes, M., Baeyen, S., Van Malderghem, C., De Vos, P., Cottyn, B., 2016. Genetic characterization of strains named as *Xanthomonas axonopodis* pv. *dieffenbachiae* leads to a taxonomic revision of the *X. axonopodis* species complex. *Plant Pathol*. 65, 792–806.

Darrasse, A., Bureau, C., Samson, R., Morris, C. E., and Jacques, M.A. 2007. Contamination of bean seed by *Xanthomonas axonopodis* pv. phaseoli associated with low bacterial densities in the phyllosphere under field and greenhouse conditions. *Eur. J. Plant Pathol*. 119:203-215.

de Sousa DM, Luis Janssen, Raphael Barboza Rosa et al. Isolation, characterization and evaluation of putative new bacteriophage species to control bacterial spot on tomato in Brazil, 27 March 2023, PREPRINT (Version 1) available at Research Square [https://doi.org/ 10.21203/rs.3.rs-2711939/v1]

Dhanvantari, B. N. 1989. Effect of seed extraction methods and seed treatments on control of tomato bacterial canker. *Can. J. Plant Pathol*. 11:400-408.

Doidge, E.M., 1920. A tomato cancer. J. Dep. Agric. Union S. Afr. 1, 718-721.

Doorn J, Ojanen-Reuhs T, Hollinger TC, Reuhs BL, Schots A, Boonekamp PM, et al. Development and application of pathovar-specific monoclonal antibodies that re- cognize the lipopolysaccharide O antigen and the Type IV fimbriae of *Xanthomonas hyacinthi*. *Appl Environ Microbiol* 1999; 65:4171–80.

Dowson D. On the systematic position and generic names of the Gram negative bacterial plant pathogens. Zbl Bakteriol Parasitenkd Infekt Hyg Abt 2 100 1939; 100:177–93.

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

Dye, D.W. (1978) Genus IX. *Xanthomonas*. Dowson 1939. N. Z. J. *Agric. Res*. 21, 153–177.

Dye, D.W., Starr, M.P. and Stolp, H. (1964) Taxonomic clarification of *Xanthomonas vesicatoria* based upon host specificity, bacteriophage sensitivity, and cultural characteristics. *Phytopathol*. Z. 51, 394–407.

El-Hendawy, H. H., Osman, M. E., and Sorour, N. M. (2005). Biological control of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* by *Rahnella aquatilis*. *Microbiological Research*, 160, 343–352.

Enman, J., Hodge, D., Berglund, K.A., Rova, U., 2008. Production of the bioactive compound eritadenine by submerged cultivation of shiitake (*Lentinus edodes*) mycelia. *J. Agric. Food Chem.* 56, 2609-2612.

EPPO, 2013a. PM 7/110 (1) *Xanthomonas* spp. (*Xanthomonas* eu*vesicatoria*, *Xanthomonas gardneri*, *Xanthomonas perforans*, *Xanthomonas vesicatoria*) causing bacterial spot of tomato and 489 sweet pepper. *EPPO Bulletin* 43: 7–20.

Fahy, P.C. and Persley, G.J. (1983). Plant bacterial diseases. A diagnostic guide. Academic Press, London, New York, Sydney pp. 393.

Feng, J., Li, J., Randhawa, P., Bonde, M., and Schaad, N. W. 2009. Evaluation of seed treatments for the eradication of *Acidovorax avenae* subsp. *citrulli* from melon and watermelon seeds. *Canadian Journal of Plant Pathology* 31(2):180-185. Online publication.

Fernandez, M., Godino, A., Príncipe, A., Morales, G.M., Fischer, S., 2017. Effect of a *Pseudomonas fluorescens* tailocin against phytopathogenic *Xanthomonas* observed by atomic force microscopy. *J. Biotechnol*. 256, 13–20.

Ferraz, H. G. M., Resende, R. S., Moreira, P. C., Silveira, P. R., Milagres, E. A., Oliveira, J. R., and Rodrigues, F. Á. (2015). Antagonistic rhizobacteria and jasmonic acid induce resistance against tomato bacterial spot. *Bragantia*, 74(4), 417–427.

Filho, R. L., Reginaldo, S. R., Alves, S., 2010: Bacterial spot and early blight biocontrol by epiphytic bacteria in tomato plants. *Pesq. Agropec. Bras., Brasília*, v.45, n.12, p.1381-1387.

Fleming CA, Trevors JT (1989) Copper toxicity and chemistry in the environment: a review. Water Air Soil Pollut., 44:143–158.

Food and Agriculture Organization of United Nations, Agriculture data (FAO), 2005 /http://www. Faostat. comS.

Fujiwara, A., Fujisawa, M., Hamasaki, R., Kawasaki, T., Fujie, M., and Yamada, T. (2011). Biocontrol of *Ralstonia solanacearum* by treatment with lytic bacteriophages. *Appl. Environ. Microbiol*., 77, 4155–4162.

Gašic, K., Ivanović, M. M., Ignjatov, M., Calić, A., and Obradović, A. 2011. Isolation and characterization of *Xanthomonas* eu*vesicatoria* bacteriophages. *Journal of Plant Pathology,* 93(2): 415-423.

Gayon U, Sauvageau C (1903) Notice sur la vie et les travaux de A. Millardet Mem la Soc des Sci Phys Nat Bordeaux 6:9–47

Giavasis, I., 2014. Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Curr. Opin. Biotech*. 26, 162e173.

Gitaitis, R., McCarter, S., and Jones, J. 1992. Disease control in tomato transplants produced in Georgia and Florida. *Plant Disease* 76(7):651-656.

Gitaitis, R., Walcott, R.R. 2007. The epidemiology and management of seed borne bacterial diseases. *Annual Review of Phytopathology* 45: 371-397.

Goncalves, E.R., and Rosato, Y.B. 2002. Phylogenetic analysis of *Xanthomonas* species based upon 16S-23S rDNA intergenic spacer sequences. *Int. J. Syst. Evol. Microbiol.* 52 2:355-361.

Graves, A., Alexander, S., 2002. Managing bacterial speck and spot of tomato with acibenzolar-S-methyl in Virginia. Online. Plant Health Prog. http://dx.doi.org/ 10.1094/PHP-2002-0220-01-RS.

Griffin K, Cherie Gambley, Philip Brown, Yujuan Li. 2017. Copper-tolerance in *Pseudomonas syringae* pv. tomato and *Xanthomonas* spp. and the control of diseases associated with these pathogens in tomato and pepper. A systematic literature review. *Crop Protection* 96 (2017) 144-150.

Grinter, R., Milner, J., Walker, D., 2012. Bacteriocins active against plant pathogenic bacteria. *Biochem. Soc. Trans.* 40, 1498–1502.

Hampton R, Ball E and De Boer S (1990) Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens, ALaboratory Manual, American Phytopathological Society, St. Paul, MN

Hamza A.A., Robène-Soustrade I., Jouen E., Gagnevin L., Lefeuvre P., Chiroleu F., Pruvost O., 2010. Genetic and pathological diversity among *Xanthomonas* strains responsible for bacterial spot on tomato and pepper in the South- west Indian Ocean Region. *Plant Disease* 94: 993-999.

Hamza, A. A., Robene-Soustrade, I., Jouen, E., Lefeuvre, P., Chiroleu, F., Fisher-Le Saux, M., Gagnevin, L. and Pruvost, O. 2012. Multilocus sequence analysis-and amplified fragment length polymorphism-based characterization of xanthomonads associated with bacterial spot of tomato and pepper and their relatedness to *Xanthomonas* species. *Syst. Appl. Microbiol*. 35:183-190.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. Trichoderma species do opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*. 2, 43-56.

Hartz, T., Miyao, G., Mickler, J., LeStrange, M., Stoddard, S., Nuñez, J., and Aegerter, B. 2008. Processing tomato production in California. UC Vegetable Production Series. http://anrcatalog.ucdavis.edu/pdf/7228.pdf

Hausbeck, M. K., Bell, J., Medina-Mora, C., Podolsky, R., and Fulbright, D. W. 2000. Effect of bactericides on population sizes and spread of *Clavibacter michiganensis* subsp. *michiganensis* on tomatoes in the greenhouse and on disease development and crop yield in the field. *Phytopathology* 90:38-44.

Hert, A.P., Marutani, M., Momol, M.T., Roberts, P.D., and Jones, J.B. 2009. Analysis of pathogenicity mutants of a bacteriocin producing *Xanthomonas perforans. Biological Control* 51 3:362-369.

Iriarte, F., Balogh, B., Momol, M., Smith, L.,Wilson, M. and Jones, J. (2007) Factors affecting survival of bacteriophage on tomato leaf surfaces. *Appl. Environ. Microbiol*. 73, 1704–1711.

Ishii, S., and Sadowsky, M.J. 2009. Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution. *Environmental Microbiology* 11 (4):733-740.

Itako, A.T., Tolentino, J.B.J., da Silva, T.A.F.J., Soman, J.M., Maringoni, A.C., 2015. Chemical products induce resistance to in tomato. *Braz. J. Microbiol*. 46, 701–706.

Jetiyanon, K., 1994. Immunization of cabbage for long-term resistance to black rot. M.S. Thesis, Auburn University, Auburn, AL, USA.

Jones J.B., Bouzar H., Stall R.E., Almira E.C., Roberts P.D., Journal Bowen B.W., Sudberry J., Strickler P.M., Chun J., 2000. Systematic analysis of xanthomonads (*Xanthomonas* spp.) associated with pepper and tomato lesions. *International Journal of Systematic and Evolutionary Microbiology* 50: 1211-1219.

Jones J.B., Lacy G.H., Bouzar H., Stall R.E., Schaad N.W., 2004. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. *Systematic and Applied Microbiology* 27: 755-762.

Jones JB, Chase AR, Harris GK. Evaluation of the biology GN MicroPlate system for identification of some plant pathogenic bacteria. *Plant Dis* 1993;77:553–8.

Jones, J.B., G.H. Lacy, and H. Bouzar. 2006. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. *Syst. Appl. Microbiol*. 29:85–86.

Jones, J.B., Jackson, L.E., Balogh, B., Obradovic, A., *et al*. 2007. Bacteriophages for Plant Disease Control. *Annu. Rev. Phytopathology* 45:245-262.

Jones, J.B., Jones, J.P., Stall, R.E., Zitter, T.A., 1991. Bacterial spot. In: Jones, J.B. et al. (Eds.), Compen- dium of Tomato Diseases. The American Phytopathological Society, St. Paul, MN, p. 73.

Joshi D., Hooda K.S., Bhatt J.C., Mina B.L., Gupta H.S., 2009. Suppressive effects of composts on soil-borne and foliar diseases of Grench bean in the field in the western Indian Himalayas. *Crop Protection* 28: 608-615.

Kaaya NKF, Mortensen CN, Mabagala RB, Massomo SMS (2003). A guide on seed borne bacterial diseases of tomato in Tanzania. Technical Bulletin, Danish Government Institute of Seed Pathology for Developing Countries (DGISP), Copenhagen, Denmark. 17p.

Kamilova, F., Lugtenberg, B., 2009: Plant-Growth-Promoting Rhizobacteria Annual Review of Microbiology, Vol. 63: 541-556

Kaur Harjinder, Leopold M. Nyochembeng, Srinivasa R. Mentreddy, Pratik Banerjee, Ernst Cebert. Assessment of the antimicrobial activity of *Lentinula edodes* against *Xanthomonas campestris* pv. *vesicatoria*. Crop Protection 89 (2016) 284-288.

Kavitha, R., Umesha, S., 2007. Prevalence of bacterial spot in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. *Crop Protection* 26, 991-997.

Kebede M, Amare A, Mohammed Y. 2013. Efficacy of Plant Extracts, Traditional Materials and Antibacterial Chemicals against *Xanthomonas campestris* pv. *vesicatoria* on Tomato Seed." *African Journal of Microbiology Research* 7(20), 2395-2400.

Keen, N. T., Dahlbeck, D., Staskwicz, B., and Belser, W. (1984). Molecular cloning of pectate lyase genes from *Erwinia chrysanthemi* and their expression in *Escherichia coli. Journal of Bacteriology*, 159,825–831.

Klein-Gordon, J. M., Xing, Y., Garrett, K. A., Abrahamian, P., Paret, M. L., Minsavage, G. V., & Vallad, G. E. (2021). Assessing changes and associations in the *Xanthomonas perforans* population across Florida commercial tomato fields via a statewide survey. *Phytopathology*®, 111(6), 1029-1041.

Klement, Z., G.L. Farkas and L. Lovrekovich. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in tobacco leaf. *Phytopath*. 54:474-477

Koenraadt H., van Betteray B., Germain R., Hiddink G., Jones J.B., Oosterhof J., Rijlaarsdam A., Rooda P., Wouldt B., 2009. Development of specific primers for the molecular detection of bacterial spot of pepper and tomato. *Acta Horticulturae* 808: 99-102.

Koike ST, Cladders P, Paulus AO (2007) Vegetable Diseases, A Color Hand- book, Academic Press, Burlington, MA, USA, 448 pp

Kotan, Recep, Ahmet Cakir, Hakan Ozer, Saban Kordali, Ramazan Cakmakci, Fatih Dadasoglu, Neslihan Dikbas, Tuba Aydin, and Cavit Kazaz. 2014. "Antibacterial Effects of Origanum Onites against Phytopathogenic Bacteria: Possible Use of the Extracts from Protection of Disease Caused by Some Phytopathogenic Bacteria." Scientia Horticulturae 172 (June): 210–20.

Kuan T.L., Minsavage G.V. (1985): Detection of *Xanthomonas campestris* pv. *carotae* in carrot seed. *Plant Disease*, 69: 758–760.

Kůdela, V., Novacky, A., Fucikovsky, L., 2002: Rostlinolékařská bakteriologie. Academia, Praha, 347 s, ISBN 80 – 200 – 0899 – 3.

Langston Jr., D. B. 2014. Diseases. Commercial Tomato Production Handbook. UGA Extension. University of Georgia, Athens, GA. Retrieved 28 August 2014 from http://extension.uga.edu/publications/files/pdf/B% 201312 _5.PDF

Lanna, R. F., Souza, R. M., Ferreira, A., Quecine, M. C., Alves, E.,andAzevedo, J. L. (2013). Biocontrol activity of Bacillus against a GFP-marked *Pseudomonas syringae* pv. tomato on tomato phylloplane. *Australasian Plant Pathology*, 42, 643–651.

Lanna-Filho, R., Souza, R. M., Marcelo, M., Villela, L., Edgar, Ribeiro-Júnior, Pedro, M., and Resende, M. L. V. (2013). Induced defense responses in tomato against bacterial spot by proteins synthesized by endophytic bacteria. *Tropical Plant Pathology*, 38(4), 295–302.

Lee, G., Lee, S. H., Kim, K. M., & Ryu, C. M. (2017). Foliar application of the leaf-colonizing yeast Pseudozyma churashimaensis elicits systemic defense of pepper against bacterial and viral pathogens. *Scientific reports*, *7*(1), 1-13.

Leite RP Jr; Jones JB; Somodi GC; Minsavage GV; Stall RE, 1995. Detection of *Xanthomonas campestris* pv. *vesicatoria* associated with pepper and tomato seed by DNA amplification. Plant Disease, 79(9):917-922 (abstract/19961000420)

Lelliott, R.A., Stead, D.E., 1987. Methods for the diagnosis of bacterial diseases of plants. In: Preece, T.F. (Ed.), Methods in Plant Pathology, vol. II. Blackwell Scientific Publications, Oxford, p. 216.

Lin, C. L., Ching, F. L., Jia, W. W., Ming, L. C., Yu, M. L., Ning, S. Y., Lowell, B., Sylvia, K. G., Jaw, F. W., Chiu, P. C., 2004: Transgenic tomato plants expressing the arabidopsis npr1 gene display enhanced resistance to a spectrum of fungal and bacterial diseases. Biomedical and life sciences, volume 13, number 6 (2004), 567- 581, doi: 10.1007/s11248-004-2375-9

Lindemann, J., 1985. Genetic manipulation of microorganisms for biological control. In: Windels, C.E., Lindow, S.E. (Eds.), Biological Control on the Phylloplane. American Phytopathological Society, St. Paul, Minnesota.

Litterick A.M., Harrier L., Wallace P., Watson C.A., Wood M., 2004. The role of un-composted materials, compost, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production: A review. Critical Re- views of Plant Sciences 23: 453-479.

Lo Cantore, P., Shanmugaiah, V., and Iacobellis, N. S. 2009. Antibacterial activity of essential oil components and their potential use in seed disinfection. *J. Agric. Food Chem*. 57:9454-9461.

Louws F.J., Fulbright D.W., Stephens C.T., de Bruijn F.J., 1995. Differentiation of genomic structure by rep-PCR fingerprinting to rapidly classify *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 85: 528-536.

Maerere, A.P., Sibuga, K.P., Mwajombe, K.K. (2006), Baseline survey report of tomato Production production in Mvomero district-Morogoro region, Tanzania, Sokoine University of Agriculture Faculty of Agriculture, Morogoro, pp. 1-31

Mallmann, W. L., and Hemstreet, C. J. (1924). Isolation of an inhibitory substance from plants. *Agricultural Research*, 28, 599-602.

Marco, G.M., and Stall, R.E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. *Plant Disease* 67:779-781.

Marcuzzo, Ll. 2009. Importância das populações epifíticas na epidemiologia de enfermidades bacterianas. *Revista de Ciências Agroveterinárias* 8: 146-151.

Mbega, E.R., Mabagala, R.B., Adriko, J., Lund, O.S., Wulff, E.G., and Mortensen, C.N. 2012. Five species of xanthomonads associated with bacterial leaf spot symptoms in tomato from Tanzania. *Plant Dis*. 96 5:760-760.

McGuire RG, Jones JB, Sasser M, 1986. Tween media for semi-selective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. *Plant Disease* 70, 887-891.

Mirik, M., Aysan, Y., & Cinar, O. (2008). Biological control of bacterial spot disease of pepper with Bacillus strains. *Turkish Journal of Agriculture and Forestry*, *32*(5), 381-390.

Mirik, M., Aysan, Y., and Cinar, O. (2008). Biological control of bacterial spot disease of pepper with Bacillus strains. *Turkish Journal of Agriculture and Forestry*, 32, 381–390.

Mohana D.C., Raveesha K.A., 2006. Anti-bacterial activity of Caesalpinia coriaria (Jacq.) Willd against plant pathogenic *Xanthomonas* pathovars: an eco-friendly approach. *Journal of Agriculture Technolology* 2: 317-327.

Momol, T., Jones, J.B., Olson, S., Obradovic, A., et al. 2002. Integrated Management of Bacterial Spot on Tomato in

Florida. In IFAS, edited by I. o. F. a. A. Sciences. Gainsville: University of Florida.

Moretti, C., Amatulli, M. T., and Buonaurio, R. 2009. PCR-based assay for the detection of *Xanthomonas* eu*vesicatoria* causing pepper and tomato bacterial spot. *The Society for Applied Microbiology, Letters in Applied Microbiology* 49:466-471.

Moss, W. P., Byrne, J. M., Campbell, H. L., Ji, P., Bonas, U., Jones, J. B., and Wilson, M. (2007). Biological control of bacterial spot of tomato using hrp mutants of *Xanthomonas campestris* pv. *vesicatoria*. *Biological Control*, 41, 199–206.

Murugaiyan, S., Bae, J. Y., Wu, J., Lee, S. D., Um, H. Y., Choi, H. K., et al. (2011). Characterization of filamentous bacteriophage PE226 infecting *Ralstonia solanacearum* strains. *J. Appl.Microbiol.* 110, 296–303.

Nakayinga, R., Makumi, A., Tumuhaise, V. *et al. Xanthomonas* bacteriophages: a review of their biology and biocontrol applications in agriculture. *BMC Microbiol* 21, 291 (2021).

Newberry, E. A., Bhandari, R., Minsavage, G. V., Timilsina, S., Jibrin, M. O., Kemble, J., et al. Independent Evolution with the Gene Flux Originating from Multiple Xanthomonas Species Explains Genomic Heterogeneity in *Xanthomonas perforans*. *Applied and Environmental Microbiology*. 85:e00885-19.

Nga NTT, Tran TN, Holtappels D, Kim Ngan NL, Hao NP, Vallino M, et al. Phage biocontrol of bacterial leaf blight disease on welsh onion caused by *Xanthomonas axonopodis* pv allii. *Antibiotics*. 2021;10:517.

Obradovic, A., Balogh, B., Olson, S.M., Jones, J.B., and Momol, M.T. 2004. Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers. *Plant Dis*. 88 7:736-740.

Obradovic, A., Jones, J. B., Momol, M. T., Olson, S. M., Jackson, L. E., Balogh, B., et al. (2005). Integration of biological control agents and systemic acquired resistance inducers against bacterial spot on tomato. *Plant Dis*. 89, 712–716.

Oliva, M., M. E. Carezzano, M. Giuliano, J. Daghero, J. Zygadlo, P. Bogino, W. Giordano, and M. Demo. 2015. "Antimicrobial Activity of Essential Oils of *Thymus Vulgaris* and *Origanum Vulgare* on Phytopathogenic Strains Isolated from Soybean." *Plant Biology* 17 (3): 758–65.

Osdaghi E, Taghavi Sm, Hamzehzarghani H And Lamichlane Jr. 2016. Occurrence and characterization of the bacterial spot pathogen *Xanthomonas* eu*vesicatoria* on pepper in Iran. *J Phytopathol* 164: 722-734.

Ozturk S, Ercisli S. (2007) Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. *Food Control* 18:535–540.

Pacumbaba, R., Beyl, C.A., Pacumbaba Jr., R., 1999. Shiitake mycelial leachate sup-presses growth of some bacterial species and symptoms of bacterial wilt of tomato and lima bean in vitro. *Plant Dis.* 83, 20-23.

Park DS, Shim JK, Kim JS, Kim BY, Kang MJ, Seol YJ, et al. PCR-based sensitive and specific detection of *Pectobacterium atrosepticum* using primers based on Rhs family gene sequences. *Plant Pathol* 2006; 55:625–9.

Park YJ, Lee BM, Hahn JH, Lee GB, Park DS. Sensitive and specific detection of *Xanthomonas campestris* pv. *campestris* by PCR using species-specific primers based on hrpF gene sequences. *Microbiol Res* 2004; 159:419–23.

Park, D.S., Shim, J.K., Kim, J.S., Lim, C.K., Shrestha, R., Hahn, J.H., Kim, H.G., 2009. Sensitive and specific detection of *Xanthomonas campestris* pv. *vesicatoria* by PCR using pathovar-specific primers based on rhs family gene sequences. *Microbiol. Res*. 164, 36-42

Parkinson, N., Cowie, C., Heeney, J., and Stead, D. (2009). Phylogenetic structure of *Xanthomonas* determined by comparison of gyr Bsequences. *International Journal of Systemic and Evolution Microbiology,* 59(264), 274.

Paul P.K., Sharma P.D., 2002. Azadirachta indica leaf extract induces resistance in barley against leaf stripe disease. *Physiological and Molecular Plant Pathology* 61: 3-13.

Pereira, R. C., Araújo, E. R., Ferreira, M. A. S. V., and Quezado-Duval, A. M. (2011). Occurrence of *Xanthomonas* species causing bacterial spot in fresh market tomato fields in Brazil. *Acta Horticulturae,* 914, 61-64.

Pernezny K, Roberts PD, Murphy JF, Golberg NP (2003) Compendium of Pepper Diseases, APS Press, St Paul, MN, USA, 63 pp

Pernezny, K., and Collins, J. 1997. Epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* on pepper: Relationships to host-plant resistance and exposure to copper sprays. Plant Dis. 81:791-794. Online publication. Publication no. D-1997- 0603-05R

Pohronezny, K., Moss, M. A., Dankers, W., and Schenk, J. 1990. Dispersal and management of *Xanthomonas campestris* pv. *vesicatoria* during thinning of direct-seeded tomato. *Plant Dis.* 74:800-805.

Potnis N, Krasileva K, Chow V, Almeida NF, Patil PB, Ryan RP, Sharlach M, Behlau F, Dow JM, Momol M, White FF, Preston JF, Vinatzer BA, Koebnik R, Setubal JC, Norman DJ, Staskawicz BJ, Jones JB. 2011 Comparative genomics reveals diversity among xanthomonads infecting tomato and pepper. *BMC Genomics* 12: 146.

Potnis N, Timilsina S, Strayer A, Shantharaj D, Barak Jd, Paret Ml, Vallad Ge And Jones Jb. 2015. Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Mol Plant Pathol* 16: 907-920.

Pradhanang P.M., Momol M.T., Olson S.M., Jones J.B., 2003. Effects of plant essential oils on Ralstonia solanacearum population density and bacterial wilt incidence in tomato. *Plant Disease* 87: 423-427.

Príncipe Analía, Maricruz Fernandez, Milenka Torasso, Agustina Godino, Sonia Fischer Effectiveness of tailocins produced by Pseudomonas fluorescens SF4c in controlling the bacterial-spot disease in tomatoes caused by *Xanthomonas vesicatoria*. *Microbiological Research* 212–213 (2018) 94–102.

Quezado-Duval A.M., Lopes C.A., Leite Júnior R.P., Lima M.F., Camargo L.E.A., 2005. Diversity of *Xanthomonas* spp. associated with bacterial spot of processing tomatoes in Brazil. *Acta Horticulturae* 695: 101-108.

Rademaker JLW, Hoste B, Louws FJ, Kersters K, Swings J, Vauterin L, Vauterin P and Bruijn FJ (2000) Comparison of AFLP and rep-PCR genomic fingerprinting with DNA–DNA homology studies: *Xanthomonas* as a model systems. *Int J Syst Evol Microbiol* 50: 665–677.

Reddy, S. A., Bagyaraj, D. J., and Kale. R. D. 2012. Management of tomato bacterial spot caused by *Xanthomonas campestris* using vermicompost. *J. Biopest* 5(1):10- 13.

Reis, F.S., Martins, A., Barros, L., Ferreira, I.C., 2012. Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between in vivo and in vitro samples. *Food Chem. Toxicol*. 50, 1201-1207

Ryan, R. P., Vorhölter, F.-J., Potnis, N., Jones, J. B., Van Sluys, M.-A., Bogdanove, A. J., and Dow J. M. 2011.

Pathogenomics of *Xanthomonas*: understanding bacterium-plant interactions. *Nature Reviews Microbiology* 9(5):344-355.

Sahin, F., and Miller, S.A. 1996. Characterization of Ohio strains of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. *Plant Dis*. 80 7:773- 778.

Saksirirat, W., Chareerak, P., & Bunyatrachata, W. (2009). Induced systemic resistance of biocontrol fungus, Trichoderma spp. against bacterial and gray leaf spot in tomatoes. *Asian J Food Agro-Ind*, *2*, S99-S104.

Sands DC (1990) Physiological criteria – determinative tests. In: Klement Z, Rudolph K and Sands D (eds) Methods in Phytobacteriology (pp 134–143) Akademiai Kiado, Budapest.

Sasser M (1990) Identification of bacteria through fatty acid analysis. In: Klement Z, Rudolph K and Sands D (eds) Methods in Phytobacteriology (pp 199–204) Akademiai Kiado, Budapest

Schaad NW, Stall RE (1988). *Xanthomonas*. In: Schaad NW, ed., Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd edn. St. Paul, MN: *American Phytopathological Society*, pp.81–94.

Schaad, N.W. and W.C. White. 1974. Survival of *Xanthomonas campestris* in soil. *Phytopathol*. 64:1518- 1520.

Schaad, N.W., J.B. Jones and W. Chun. 2001. Laboratory guide for identification of plant pathogenic bacteria. 3rd Ed. APS Press. Minnesota.

Schlechter, R. O., and Remus-Emsermann, M. N. 2019. Delivering & quot; Chromatic Bacteria & quot; Fluorescent Protein Tags to Proteobacteria Using Conjugation. *Bioprotocol*. 9:e3199–e3199.

Schwartz, A. R., Potnis, N., Timilsina, S., Wilson, M., Patané, J., Martins, Jr. Staskawicz, B. J. (2015). Phylogenomics of *Xanthomonas* field strains infecting pepper and tomato reveals diver- sity in effector repertoires and identifies determinants of host specificity. *Frontiers in Microbiology*, 6, 535–00.

Scott J.W., Jones J.B. And G.C. Somodi., 1995. Screening Tomato Accessions for Resistance to *Xanthomonas campestris*pv. *vesicatoria*, Race T3. Hortscience, *Hort Science* June 1995 vol. 30 no. 3 579-581

Shenge, K. C., Mabagala, R. B., & Mortensen, C. N. (2007). Identification and characterization of strains of Xanthomonas campestris pv. Vesicatoria from Tanzania by biolog system and sensitivity to antibiotics. *African Journal of Biotechnology*, *6*(1), 15.

Shenge, K. C., Mabagala, R. B., & Mortensen, C. N. (2010). Current status of bacterial-speck and-spot diseases of tomato in three tomato-growing regions of Tanzania. *Journal of Agricultural Extension and Rural Development*, *2*(5), 84-88.

Sijam, K., Chang, C. J., and Gitaitis, R. D. 1991. An agar medium for the isolation and identification of *Xanthomonas campestris* pv. *vesicatoria* from seed. *Phytopathology* 81:831-834.

Sijam, K., Chang, C.J. and Gitaitis, R.D. 1992 A medium for differentiating tomato and pepper strains of *Xanthomonas campestris* pv. *vesicatoria*. *Can. J. Plant Pathol*. 14, 182–184.

Sikora, E. J., 1994, Bacterial wilt of tomatoes, Plant disease Notes, Extension systém, ANR-862 Bacterial wilt of tomatoes.

Silverman, A. I., Peterson, B. M., Boehm, A. B., McNeill, K., and Nelson, K. L. 2013. Sunlight inactivation of human viruses and bacteriophages in coastal waters containing natural photosensitizers. *Environmental Science and Technology* 47(4):1870-1878.

Solomakos, N., Govaris, A., Koidis, P., & Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin and their combination against Escherichia coli O157: H7 in minced beef during refrigerated storage. *Meat science*, *80*(2), 159-166.

SommerAL 1931. Copper as an essential for plant growth. *Plant Physiol* 6:339–345

Stall, R.E., Beaulieu, C., Egel,D., Hodge,N.C., Leite, R.P., Minsavage,G.V., Bouzar, H., Jones, J.B., Alvarez, A.M. and Benedict, A.A. (1994) Two genetically diverse groups of strains are included in *Xanthomonas campestris* pv. *vesicatoria*. *Int. J. Syst. Bacteriol*. 44, 47–53.

Stall, R.E., Jones, J.B., Minsavage, G.V., 2009. Durability of resistance in tomato and pepper to xanthomonads causing bacterial spot. *Annu. Rev. Phytopathol*. 47, 265e284.

Sticher, L., Mauch-Mani, B., & Métraux, A. J. (1997). Systemic acquired resistance. *Annual review of phytopathology*, *35*(1), 235-270.

Stockwell, V. O., & Duffy, B. (2012). Use of antibiotics in plant agriculture. *Revue Scientifique Et Technique-Office International Des Epizooties*, *31*(1).

Stoyanova, M., Vancheva, T., Moncheva, P. and Bogatzevska, N. 2014. Differentiation of *Xanthomonas* spp. causing bacterial spot in Bulgaria based on biolog system. *Int. J. Microbiol*. 2014:495476.

Strayer-Scherer, A., Liao, Y. Y., Abrahamian, P., Timilsina, S., Paret, M., Momol, T. & Vallad, G. E. (2019). Integrated management of bacterial spot on tomato in Florida. EDIS, 6, 8.

Suárez-Estrella F., Bustamante M.A., Moral R., Vargas-García M.C., López M.J., Moreno J., 2012. In vitro control of Fusarium wilt using agroindustrial subproduct-based com-posts. *Journal of Plant Pathology* 94: 59-70.

Sun, X., Nielsen, M. C., and Miller, J. W. 2002. Bacterial spot of tomato and pepper. Plant Pathology Circular No. 129 (revised). Florida Department of Agriculture and Conservation Services.

Sundin, G. W., Castiblanco, L. F., Yuan, X., Zeng, Q., and Yang, C.-H. (2016). Bacterial disease management: challenges, experience, innovation, and future prospects. *Mol Plant Pathol*. 2016 Dec; 17(9):1506-1518.

Sutic, D. 1957. Tomato bacteriosis. Posebna Izd.Inst.Zasht.Bilja, Beograd [Spec. Edit.Inst.Plant Prot., Beograd]. 6:65.

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. and 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.

Udayashankar, A., Nayaka, S.C., Reddy, M., Srinivas, C., et al., 2011. Plant growth- promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Biol. Control* 59, 114-122.

United States Environmental Protection Agency, 2016. New Biopesticide Active In- gredients-2010. www3.epa. gov/pesticides/biopesticides/product_lists/new_ai_ 2010. html/ (Accessed 31.05.16.).

Vale, F. X. R., Zambolim, L., Zambolim, E. M., and Alvarenga, M. A. R. (2004). Manejo integrado das doenças do tomateiro: Epidemiologia e Controle. In M. A. R. Alvarenga (Ed.), Tomate: produção em campo, em casa-de-vegetação e em hidroponia (pp. 217-302). Lavras: Editora UFLA.

Vallad, G. E., & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop science*, *44*(6), 1920-1934.

Vasinauskiene M, Radusiene J, Zitikaite I, Surviliene E. (2006) Anti- bacterial activities of essential oil from aromatic and medicinal plants against growth of phytopathogenic bacteria. Agro Res 4:437–440.

Vauterin, L., Hoste, B., Kersters, K., Swings, J., 1995. Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol*. 45, 472e489.

Venette JR, Lamey HA, Smith RC (1996). Bacterial spot and bacterial speck of Tomato. North Dakota State University Extension Services. p. 2.

Vera, Cruz CMV, Gossele F, Kersters K, Segers P, Van den Mooter M, Swings J, et al. Differentiation between *Xanthomonas campestris* pv. oryzae, *Xanthomonas oryzae* pv. *oryzicola* and the bacterial brown blotch pathogen on rice by numerical analysis of phenotypic features and protein gel electrophoregrams. *J Gen Microbiol* 1984;130: 2983–99.

Versalovic, J., Schneider, M., De Bruijn, F. J., and Lupski, J.R. 1994. Genomic Fingerprinting of Bacteria Using Repetitive Sequence-Based Polymerase Chain Reaction. *Methods in Molecular and Cellular Biology* 5:25-40.

Wang, S.-C., Chen, A.-P., Chou, S.-J., Kuo, C.-H., and Lai, E.-M. 2023. Soil Inoculation and Blocker-Mediated Sequencing Show Effects of the Antibacterial T6SS on Agrobacterial Tumorigenesis and Gallobiome. mBio. 0:e00177-23

Wilson, M., Moss, W.P., Ji, P., Wang, S.-Y., Dianese, A.C., Zhang, D., Campbell, H.L., 1998. Molecular approaches in the development of biocontrol agents of foliar and floral bacterial pathogens. In: Duffy, B.K., Rosenberger, U., Defago, G. (Ed.), *Molecular Approaches in Biological Control*, IOBC/wprs Bulletin No. 21(9) pp. 247–255.

Wulff, E. G., Mguni, C. M., Mansfeld-Giese, K., Fels, J., Lubeck, M., and Hockenhull, J. (2002). Biochemical and molecular characterization of Bacillus amyloliquefaciens, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris*. *Plant Pathology*, 51, 574–584.

Yang W, Francis DM. 2005. Marker-assisted selection for combining resistance to bacterial spot and bacterial speck in tomato. *J. Amer. Soc. Hort. Sci*. 130: 716-721.

Yang, W., Sacks, E. J., Lewis Ivey, M. L., Miller, S. A., and Francis, D. M. 2005. Resistance in *Lycopersicon esculentum* intraspecific crosses to race T1 strains of *Xanthomonas campestris* pv. *vesicatoria* causing bacterial spot of tomato. *Phytopathology* 95:519-527.

Yim, W. J., Kim, K. Y., Lee, Y. W., Sundaram, S. P., Lee, Y., and Sa, T. M. (2014). Real time expression of ACC oxidase and PR-protein genes mediated by Methylobacterium spp. in tomato plants challenged with *Xanthomonas campestris* pv. *vesicatoria*. *Journal of Plant Physiology*, 171, 1064–1075.

Young, J.M., Park, C.D., Shearman, H.M., Fargier, E., 2008. A multilocus sequence analysis of the genus *Xanthomonas*. *Syst. Appl. Microbiol*. 31, 366–377.