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Full Length Research Paper

Detection and identification of *Xanthomonas axonopodis* pv. *phaseoli* on bean seed collected in Serbia

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Laboratory assays were conducted to detect and identify *Xanthomonas axonopodis* pv. *phaseoli* on bean seeds collected from naturally contaminated commercial crops. Semi-selective media*, viz.* *Xanthomonas campestris* pv*. phaseoli* agar and milk tween agar were used to detect the bacterium from whole bean seed extract. Colonies of the bacterium were yellow, mucoid and convex on these media and zones of hydrolysis formed around them. The pathogenicity of the strains was confirmed by stem inoculation of young bean plants. Identification of the strains was proved by using rapid methods (ELISA and PCR). The results showed the presence of seed-borne *X. axonopodis* pv. *phaseoli* in 20 out of the 23 assayed bean cultivars that are commercially grown in Serbia. These results indicate that the seed of the bean cultivars grown in our country is highly infected by the bacterium.

Key words: Common bacterial blight, detection, identification, seed-borne.

INTRODUCTION

Common bacterial blight of bean (*Phaseolus vulgaris* L.) is caused by the seed-borne bacteria *Xanthomonas axonopodis* pv. *phaseoli* (Smith) (Vauterin et al., 1995) and *X. axonopodis* pv. *phaseoli* var. *fuscans* (Burkholder) Starr and Burkholder, as the brown-pigmented variant (Leben, 1981; Schaad, 1982). This bacterium is a major pathogen of common bean all over the world (Mabagala and Saettler, 1992; Opio et al., 1996).). The disease causes yield losses ranging between 10 and 40%, depending on bean cultivar susceptibility and environmental conditions (Saettler, 1989). Common blight has been reported in most bean-producing areas of Serbia as one of the major limiting factors in bean production (Todorović, 2006; Popović et al., 2007; Popović, 2008). The management of common bacterial blight is difficult and is based mainly on pathogen-free seed and resistant cultivars (Zaumeyer and Thomas, 1957).

Seed transmission is the primary means by which the pathogen is disseminated (Cafati and Saettler, 1980; Weller and Saettler, 1980). Internally and externally infested seeds are important sources of primary inocula for *X. a. pv. phaseoli* (Weller and Saettler, 1980; Hall, 1994). Approximately 1 in 10000 seeds is capable of causing an outbreak of blight (Sutton and Wallen, 1970), or 1000 to 10000 bacteria per seed is the minimum needed to produce infected plants under field conditions (Weller and Saettler, 1980).

Although several serological (Malin et al., 1983; Sheppard et al., 1989), specific phages (Katznelson et al., 1954) and polymerase chain reaction methods (Audy et al., 1996; Molouba et al., 2001) have been developed for detection of this pathogen in seed, isolation on semi-selective media remains the most widely used detection method (Remeeus and Sheppard, 2006; ISF, 2006; Sheppard et al., 2007; Balaž et al., 2008).

In Serbia, commercial bean grain is used for planting since native varieties predominate in the production. Certified seed is seldom used even in the case of the new domestic cultivars for which seed production has been organized. Certified seed is typically used in the first

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production year only (Todorović et al., 2008). In the light of the above, we carried out this assay to detect and identify *X. a.* pv. *phaseoli* on bean seed lots collected from commercial crops in Serbia. The method involved extraction of bacterium from the whole seed, isolation on semi-selective media and determination of pathogenicity of the obtained strains. Using the rapid methods (ELISA and PCR), identification of strains was confirmed.

MATERIALS AND METHODS

Working samples of bean seed lots were obtained from Institute of Field and Vegetable Crops in Novi Sad. Seeds of 23 bean cultivars (Balkan, Belko, C 20, Dobrudzanski rani, Dobrudzanski rani 7, Dvadesetica, Galeb, HR 45, KB 100, KB 101, Maxa, Medijana, Naya Nayahit, Oplenac, Oreol, Panonski gradistanac, Panonski tetovac, Slavonski zutozeleni, Sremac, Xan 159, Xan 208, Xan 273 and Zlatko) were collected from naturally contaminated commercial crops. Five 1000-seed sub-samples were gathered for each cultivar. There was a total of 115 sub-samples.

Pure cultures of *X. axonopodis* pv. *phaseoli* reference strains of the non fuscans type - GSPB 1241 (Goettinger Sammlung Phytopathogener Bakterien, Deutschland) and fuscans type - CFBP 6165 (La Collection Française de Bactéries Phytopathognes, France) were used in all assays.

Extraction of bacteria from the seed

The seeds were first washed in running water and then placed on sterile laboratory paper to dry. After that, sub-samples were weighed. Each sub-sample was soaked in seed extraction solution (0.85% saline with Tween 20) in the proportion of 1:2 (1 g of seed in 2 ml of solution) and kept overnight at 5°C.

Isolation on semi-selective media

After incubation, 10-fold dilution series (to 10^{-5}) of the seed extract was prepared. Afterwards, 0.1 ml of each undiluted and diluted extracts were spread on two plates of two semi-selective media, *Xanthomonas campestris* pv. *phaseoli* Agar (XCP1) and Milk Tween Agar (MT), described by McGuire et al. (1986) and Goszczynska and Serfontein (1998), respectively.

Pure cultures of *X. a.* pv. *phaseoli* reference strains (GSPB 1241, CFBP 6165) were also spread on both semi-selective media. The plates were incubated at 28°C for five days. Then, the sample plates were visually assessed for the presence of colonies showing typical *X. axonopodis.* pv. *phaseoli* morphology and compared with the reference strains. Suspected colonies as well as the reference strains were transferred on to yeast dextrose chalk medium (YDC) (Schaad, 1988) and incubated for 2-3 days at 27°C. The transferred colonies were again compared with the reference strains. Two typical strains for each medium were selected for further investigation (80 representative strains). The representative strains used in the study are listed in Table 1.

Pathogenicity test

The strains were checked for pathogenicity using young bean plants: Zlatko, a susceptible bean variety, was planted in sterilized soil and incubated in a growth chamber at 25°C till first visible leaf appeared (10 days after sowing). Plants were sprayed with water two hours before inoculation to provide favourable conditions for infection.

Inoculation of plants was made with a sterile toothpick, which was dipped into the bacterial culture of investigated strains on the YDC medium. The toothpick was pushed in until its tip emerged on the other side of the node. After that, the toothpick was turned slightly while being pulled out in order to release the bacteria. Four plants were inoculated per strain. The positive (reference cultures) and negative (sterile water) controls were prepared in a similar manner.

Seedlings were placed in a growth chamber at 25°C with 80% relative humidity and adequate light for plant growth. Symptoms were recorded after 4-5, 8-10 and 14 days and compared with the positive and negative controls. Pathogenicity was evaluated on the inoculated seedlings based on necrosis intensity on stems and occurrence of typical symptoms on leaves.

Serological and molecular identification

In addition to colony morphology on XCP1 and MT and pathogenicity proved on young bean plants, ELISA (enzyme-linked immunosorbent assay) and PCR (Polymerase Chain Reaction) assay were conducted to confirm the identity of all investigated strains.

ELISA

Double-antibody sandwich (DAS)-ELISAs and plate trapped antigen (PTA)-ELISAs were performed using commercial kits (Loewe Biochemica GmbH, Germany for DAS ELISA; ADGEN Phytodiagnostics, Neogen Europe Ltd., Scotland, U.K. for PTA ELISA) and following the manufacturers' instructions. Bacterial suspensions were prepared in sterile water with pure bacterial cultures grown on the YDC medium for 48 h at 27°C. Reference strains of *X. a.* pv. *phaseoli* (GSPB 1241, CFBP 6165) were used as positive controls, while a reference strain of *Erwinia amylovora* (NCPPB 595) was used as the negative control.

PCR

PCR amplifications were conducted with DNA extracted from pure bacterial cultures. Cultures were grown on the YDC medium at 27°C for 48 h and cells from 0.4 ml of water suspension (3×10^8 cfu/ml) were used for DNA extraction. Bacterial suspensions were centrifuged at 11000 rpm for 1 min and supernatant was used for individual PCR assays. Reference strains of *X. axonopodis.* pv. *phaseoli* (GSPB 1241, CFBP 6165) were used as positive controls and a reference strain of *E. amylovora* (NCPPB 595) was used as the negative control.

To identify representative strains, polymerase chain reaction (PCR) was preformed with the *X. axonopodis* pv. *phaseoli* specific primer pair, X4c (5´-GGC AAC ACC CGA TCC CTA AAC AGG -3´) and X4e (5´-CGC CCG GAA GCA CGA TCC TCG AAG -3´) (Audy et al., 1994; Schaad et al., 2001), which directs the amplification of the 730-bp DNA fragment.

The PCR amplification assay was performed in a 25 µl reaction mixture containing Taq DNA polymerase 1.25 U, 50 mM KCl, 30 mM Tris-HCl, 1.5 mM Mg^{2+}, 0.1% Igepal-CA630, 200 µM dNTP, 0.8 µM of primers and 2 µl of DNA. A Mastercycler ep gradient S (Eppendorf, Germany) was used for PCR assay applying the following thermal profile amplifications: initial denaturation at 94°C for 3 min (single-step reaction), followed by 30 repeated cycles of melting, annealing and DNA extension at 94°C for 1 min, 65°C for 1 min and 72°C for 2 min, respectively. Final extension time was 5 min at 72°C (single-step reaction). The amplified DNA fragments were electrophoresed in 2.0% agarose gel in 1 × TBE buffer and

Table 1. Isolation from bean seed collected from commercial crops (naturally contaminated).

Strains	Bean variety	Media	Strains	Bean variety	Media
TX113	Dvadesetica	MT	TX231	Naya Nayahit	MT
TX128	Dvadesetica	MT	TX232	Nya Nayahit	MT
TX105	Dvadesetica	XCP1	TX233	Naya Nayahit	XCP1
TX111	Dvadesetica	XCP1	TX234	Naya Nayahit	XCP1
TX150	Oplenac	MT	TX235	Panonski tetovac	MT
TX153	Oplenac	MT	TX236	Panonski tetovac	MT
TX160	Oplenac	XCP1	TX237	Panonski tetovac	XCP1
TX252	Oplenac	XCP1	TX238	Panonski tetovac	XCP1
TX200	Slavonski zutozeleni	MT	TX241	Dobrudzanski rani 7	MT
TX201	Slavonski zutozeleni	MT	TX242	Dobrudzanski rani 7	MT
TX203	Slavonski zutozeleni	XCP1	TX244	Dobrudzanski rani 7	XCP1
TX204	Slavonski zutozeleni	XCP1	TX245	Dobrudzanski rani 7	XCP1
TX209	Balkan	MT	TX254	Xan 159	MT
TX210	Balkan	MT	TX255	Xan 159	MT
TX212	Balkan	XCP1	TX256	Xan 159	XCP1
TX213	Balkan	XCP1	TX257	Xan 159	XCP1
TX214	Sremac	MT	TX258	Xan 208	MT
TX215	Sremac	MT	TX259	Xan 208	MT
TX216	Sremac	XCP1	TX260	Xan 208	XCP1
TX217	Sremac	XCP1	TX261	Xan 208	XCP1
TX179	Belko	MT	TX262	Xan 273	MT
TX180	Belko	MT	TX263	Xan 273	MT
TX183	Belko	XCP1	TX264	Xan 273	XCP1
TX184	Belko	XCP1	TX265	Xan 273	XCP1
TX205	Zlatko	MT	TX266	C 20	MT
TX206	Zlatko	MT	TX267	C 20	MT
TX207	Zlatko	XCP1	TX268	C 20	XCP1
TX208	Zlatko	XCP1	TX269	C 20	XCP1
TX218	Panonski gradistanac	MT	TX270	KB 100	MT
TX219	Panonski gradistanac	MT	TX271	KB 100	MT
TX220	Panonski gradistanac	XCP1	TX272	KB 100	XCP1
TX221	Panonski gradistanac	XCP1	TX273	KB 100	XCP1
TX222	Maksa	MT	TX275	KB 101	MT
TX223	Maksa	MT	TX276	KB 101	MT
TX224	Maksa	XCP1	TX277	KB 101	XCP1
TX225	Maksa	XCP1	TX278	KB 101	XCP1
TX226	Galeb	MT	TX279	Oreol	MT
TX227	Galeb	MT	TX280	Oreol	MT
TX229	Galeb	XCP1	TX283	Oreol	XCP1
TX230	Galeb	XCP1	TX284	Oreol	XCP1

visualized with ultraviolet light after ethidium bromide staining.

RESULTS

Isolation on semi-selective media

Seed samples of 20 bean varieties (Balkan, Belko, C 20, Dobrudzanski rani 7, Dvadesetica, Galeb, KB 100, KB 101, Maxa, Naya Nayahit, Oplenac, Oreol, Panonski gradistanac, Panonski tetovac, Slavonski zutozeleni, Sremac, Xan 159, Xan 208, Xan 273 and Zlatko) were infected with *X. axonopodis* pv. *phaseoli*. Not a single suspected bacterium colony formed on three bean varieties (Dobrudzanski rani, HR 45 and Medijana).

On XCP1 and MT media, individual colonies of *X. axonopodis* pv. *phaseoli* were formed in 10^{-2} to10^{-5} dilutions after 4-5 days of incubation (Figures 1 and 2).

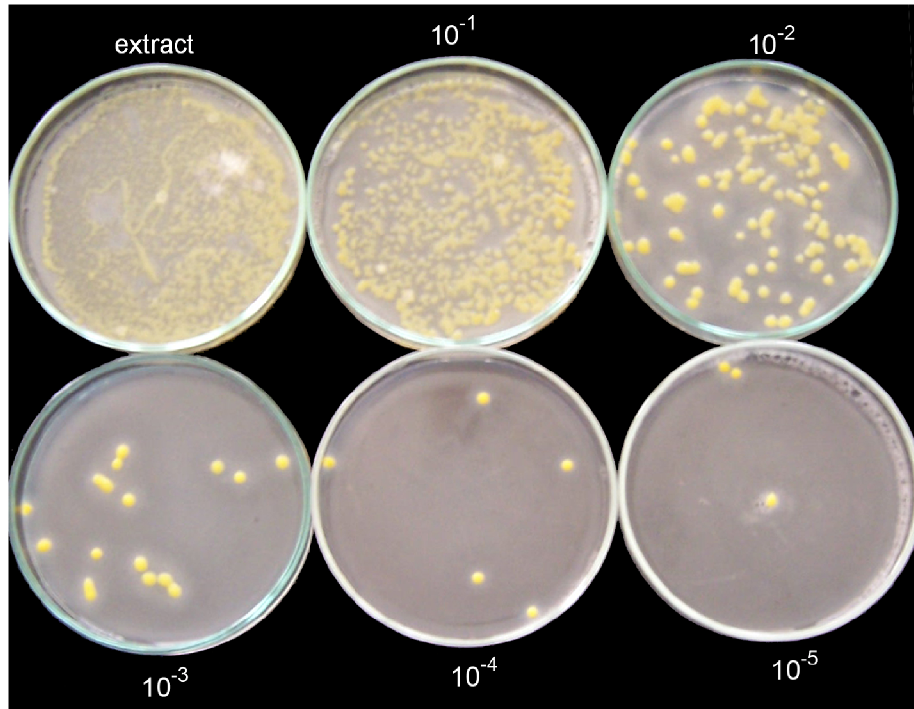

Figure 1. View of bacteria colonies from bean variety sremac on XCP1 medium, different dilutions.

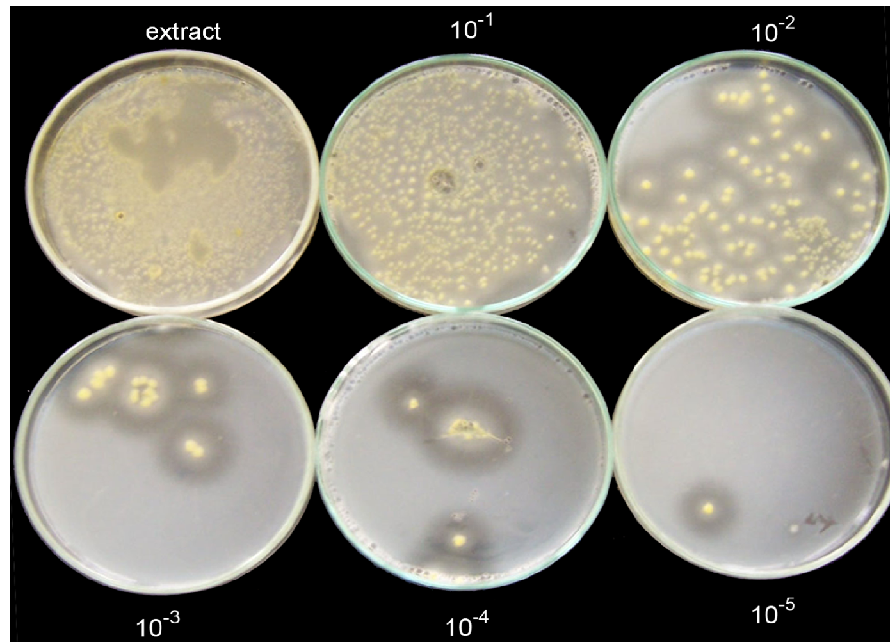

Figure 2. View of bacteria colonies from bean variety Sremac on MT medium, different dilutions.

They were yellow, convex and mucoid and differed in colony size (1 - 3 mm). On XCP1, colonies were surrounded by a clear zone of starch hydrolysis (Figure 1). On MT, colonies were distinguished by two zones of hydrolysis; a large, clear zone of casein hydrolysis and a smaller, milky zone of Tween 80 hydrolysis (Figure 2). The *X. axonopodis* pv. *phaseoli* colonies grown on YDC were yellow and mucoid. Colonies of *X. axonopodis* pv.

Figure 3. Symptoms on inoculated plant of bean, strain TX278

phaseoli var. *fuscans* were formed on three samples (bean varieties Belko, Galeb and Oreol). They developed brown pigment after more than 5 days of incubation.

Pathogenicity test

The presence of suspect strains (Table 1) was confirmed by pathogenicity. Typical symptoms attributable to *X. axonopodis* pv. *phaseoli* symptoms were formed after 4 - 5 days on inoculated young bean plants. Those were dark green water-soaked lesions at the point of entry of the toothpick. The lesions became red-brown and elongated and extended into the stem, causing slight to severe stem cracking (Figure 3).

Leaf chlorosis and necrosis occurred after 7 - 8 days. Wilting and flagging of the top foliage followed by necrosis occurred 14 - 18 days after inoculation. After 20 days, most young bean plants were decayed.

Serological and molecular identification

ELISA

Strains were tested by DAS- and PTA-ELISA using commercial antibodies available for *X. axonopodis* pv. *phaseoli* detection and using two strains of *X. axonopodis* pv. *phaseoli* as positive controls. The used polyclonal antibody reacted as expected with all the strains, producing clear positive reactions. Our results, therefore, indicate that strains of *X. axonopodis* pv. *phaseoli* can be detected using commercial ELISA tests.

PCR

The X4c and X4e primer pair directed the amplification of the 730 bp target DNA fragment from all the *X. axonopodis* pv. *phaseoli* strains tested. These results

confirmed that the strains were *X. axonopodis* pv. *phaseoli* and that the X4c and X4e primer pair has the capacity to direct the amplification of the target fragment from *X. axonopodis* pv. *phaseoli* strains.

DISCUSSION

X. axonopodis pv. *phaseoli* is seed-borne, carried internally and externally on bean seed (Weller and Saettler, 1980; Hall, 1994). Seed-borne inoculum is a major agent of bacterial survival and dissemination (Cafati and Saettler, 1980). Presence of diseased plants in seed fields affects the certification eligibility of the crop, as defined by certification rules and regulations (Frank, 1998). For the above reasons, many different diagnostic methods have been developed for detection of *X. axonopodis* pv. *phaseoli* on bean seeds, but isolation on semi-selective media is the most widely used one (NSHS, 2002; Remeeus and Sheppard; 2006; ISF, 2006; Sheppard et al., 2007). Our assays, based on isolation on two semi-selective media, MT and XCP1, were able to detect *X. axonopodis* pv. *phaseoli* on bean seed. The bacterium *X. axonopodis* pv. *phaseoli* can be successfully isolated by plating the extract obtained from the whole seeds onto the semi-selective media MT and XCP1. After 4 - 5 days, colonies of the bacterium are clearly visible on these media (yellow, mucoid and convex) and zones of hydrolysis form around them (NSHS, 2002; ISF, 2006; Remeeus and Sheppard, 2006; Sheppard et al., 2007). MT is a useful medium for semi-selective isolation and for preliminary distinction between *X. axonopodis.* pv. *phaseoli* and the other two seed-borne pathogens of bean, *Pseudomonas savastanoi* pv. *phaseolicola* (halo blight) and *P. syringae* pv. *syringae* (bacterial brown spot) (Goszczynska and Serfontein, 1998). Other useful semi-selective media for the isolation of *X. axonopodis* pv. *phaseoli* are MXP (Claflin et al., 1987), M-SSM (Mabagala and Saettler, 1992) and YSSM-XP (Dhanvantari and Brown, 1993). Starch is frequently included in the medium to help detect *X. axonopodis.* pv. *phaseoli* because it hydrolyses starch, which is uncommon because most phytopathogenic bacteria do not hydrolyze starch.

The isolation procedure and identification may be carried out using different techniques such as cultural and biochemical tests, bacteriophage, serology (agglutination, gel diffusion, IF, ELISA), host inoculation (leaves, pods, stems) (Sheppard et al., 1989) and molecular assays (Audy et al., 1994, 1996). In this study, pathogenicity of the isolates was confirmed by stem inoculation of young bean plants and formation of dark green elongated spots and cracking of the tissue. These symptoms are described in Sheppard et al. (1989, 2007) and ISF (2006). Various diagnostic physiological tests are available for use with pure cultures but pathogenicity test is required for detection at the pathovar level (Sheppard et al., 1989). The seed-soak wash can also be directly injected into susceptible plants or vacuum-infiltrated into pre-germinated bean seeds, which are then observed for typical common blight symptoms (Venette et al., 1987; Sheppard et al., 1989).

The rapid methods (ELISA and PCR) provided definitive evidence that the strains obtained from the bean seeds were the bacterium *X. axonopodis* pv. *phaseoli.* The most reliable way for detection and identification of phytopathogenic bacteria on seeds is a combination of classic and rapid methods. Immunoassays are available for identifying *X. axonopodis* pv. *phaseoli* (Malin et al., 1983; Roth, 1988). Specific diagnosis of *X. axonopodis* pv. *phaseoli* was achieved with a DNA hybridization probe (Gilbertson et al., 1989). A higher level of sensitivity was achieved by a PCR-based assay with specific primer pair X4c and X4e that was specific for *X. axonopodis* pv. *phaseoli* (Audy et al., 1994). The PCR-based assay could be used to detect DNA content in as little as 1 c.f.u. of *X. axonopodis* pv. *phaseoli* (Audy et al., 1996).

In this study, we tested bean seeds from naturally infected commercial crops (23 cultivars). The experiments confirmed the presence of *X. a.* pv. *phaseoli* on 20 of the 23 cultivars tested, indicating that the seed of the bean cultivars grown in Serbia is infected by the bacterium to a high degree. Based on these results, the two semi-selective media, MT and XCP1, are recommended for routine testing of *X. axonopodis* pv. *phaseoli* on bean seed.

REFERENCES

Audy P, Braat CE, Saindon G, Huang HC, Laroche A (1996). A rapid and sensitive PCR-based assay for concurrent detection of bacteria causing common and halo blights in bean seed. Phytopathology 86(4): 361-366.

Audy P, Laroche A, Saindon G, Huang HC, Gilbertson RL (1994). Detection of the bean common blight bacteria, *Xanthomonas campestris* pv. *phaseoli* and *X. c. phaseoli* var. *fuscans*, using the polymerase chain reaction. Phytopathology 84(10): 1185-1192.

Balaž J, Popović T, Vasić M, Nikolić Z (2008). Razrada metoda za dokazivanje *Xanthomonas axonopodis* pv. *phaseoli* na semenu pasulja. Pestic. Fitomed., 23(2): 89-98.

Cafati CR, Saettler AW (1980). Transmission of *Xanthomonas phaseoli* in seeds of resistant and susceptible *Phaseolus* genotypes. Phytopathology 70(7): 638-640.

Claflin LE, Vidaver AK, Sasser M (1987). MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology 77(5): 730-734.

Dhanvantari BN, Brown RJ (1993). YSSM-XP medium for *Xanthomonas campestris* pv. *phaseoli*. Can. J. Plant Pathol., 15(3): 168–174.

Franc GD (1998). Bacterial Diseases of Beans. Wyoming, U.S.: Cooperative Extension Service, University of Wyoming.

Gilbertson RL, Maxwell DP, Hagedorn, DJ, Leong SA (1989). Development and application of a plasmid DNA probe for detection of bacteria causing common bacterial blight of bean. Phytopathology 79(5): 518-525.

Goszczynska T, Serfontein JJ (1998). Milk Tween Agar, a Semiselective Medium for Isolation and Differentiation of *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*. J. Microbiol. Meth., 32(1): 65-72.

Hall R (1994). Compendium of Bean Diseases. New York, USA: The

American Phytopathological Society.
ISF - International Seed Testing (2006). Method for the Detection of *Xanthomonas axonopodis* pv. *phaseoli* on Bean Seed. Nyon, Switzerland [http://www.worldseed.org/cms/medias/file/TradeIssues/Phytosanitary Matters/SeedHealthTesting/ISHI-Veg.
Katznelson H, Sutton MD, Bayley ST (1954). The use of bacteriophage of *Xanthomonas phaseoli* in detecting infection in beans, with observations on its growth and morphology. Can. J. Microbiol., 1(1): 22-29.
Leben C (1981). Bacterial pathogens: Reducing Seed and In Vitro Survival by Physical Treatments. Plant Dis., 65: 876-878.
Mababgala RB, Saettler AW (1992). An improved semi-selective media for the recovery of *Xanthomonas campestris* pv *phaseoli*. Plant Dis., 76: 443-446.
Malin EM, Roth DA, Belden EL (1983). Indirect immunofluorescent staining for detection and identification of *Xanthomonas campestris* pv. *phaseoli* in naturally infected bean seed. Plant Dis., 67: 645-647.
McGuire RG, Jones JB, Sasser M. (1986). Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. Plant Dis., 70: 887-891.
Molouba F, Guimier C, Berthier C, Guenard M, Olivier V, Baril C, Horvais A (2001). Detection of bean seed-borne pathogens by PCR. Acta Horticult., 546: 603-607.
NSHS - National Seed Health System (2002). Seed Health Testing and Phytosanitary Field Inspection Methods Manual. Reference Manual B (RM-B) [http://www.seedhealth.org/files/pdf/RMB0402.pdf.
Opio AF, Allen DJ, Teri JM (1996). Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. Plant Pathol., 45: 1126-1133.
Popović T (2008). Detekcija fitopatogenih bakterija na semenu pasulja i osetljivost sorti. Novi Sad, Serbia: University of Novi Sad, PhD thesis.
Popović T, Balaž J, Vasić M (2007). Rasprostranjenost bakteriozne plamenjače pasulja (*Xanthomonas axonopodis* pv. *phaseoli*) u Vojvodini. Zlatibor, Serbia: XIII Simpozijum sa savetovanjem o zaštiti bilja sa međunarodnim učešćem, pp. 87-88.
Remeeus PM, Sheppard JW (2006). Proposal for a new method for detecting *Xanthomonas axonopodis* pv. *phaseoli* on bean seeds. Bassersdorf, Switzerland: ISTA Method Validation Reports 3, pp. 1-11.
Roth DA (1988). Development of bean seed assays for bacterial pathogens. Ann. Rep. Bean Improv. Coop., 31: 76-77.
Saettler AW (1989). Assessment of yield loss caused by common blight of beans in Uganda. Ann. Rep. Bean Improv. Coop. 35: 113-114.
Schaad NW (1982). Detection of Seedborne Bacterial Plant Pathogens. Plant Dis., 66: 885-890.
Schaad NW (1988). Laboratory Guide for Identification of Plant Pathogenic Bacteria. Minnesota, U.S.: The American Phytopathological Society.
Sheppard JW, Kurowski C, Remeeus PM (2007). International Rules for Seed Testing, 7-021: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* on *Phaseolus vulgaris*. Bassersdorf, Switzerland: International Seed Testing Association (ISTA).
Sheppard JW, Roth DA, Saettler AW (1989). Detection of *Xanthomonas campestris* pv. *phaseoli* in Bean. In: Saettler AW, Schaad NW, Roth DA (eds) Detection of Bacteria in Seed and Other Planting Material. Minnesota: U.S.: The American Phytopathological Society, pp. 17-29.
Sutton MD, Wallen VR (1970). Epidemiological and ecological relations of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* on beans in south western Ontario, 1961-1968. Can. J. Bot., 48(7): 1329-1334.
Todorović B (2006). Bakteriološke karakteristike *Xanthomonas campestris* pv. *phaseoli*, patogena pasulja u Srbiji i mogućnosti suzbijanja. Novi Sad, Serbia: University of Novi Sad, PhD thesis.
Todorović J, Vasić M, Todorović J, eds. (2008). Pasulj i boranija. Novi Sad, Serbia: Institute for vegetable and field crops; Banja Luka, Bosnia and Herzegovina: Faculty of Agriculture.
Vauterin L, Hoste B, Kersters K, Swings J (1995). Reclassification of Xanthomonas. Internat. J .Syst .Bacteriol. 45: 472–489.
Venette JR, Lamppa RS, Albaugh DA, Nayes JB (1987). Presumptive procedure (dome test) for detection of seedborne bacterial pathogens in dry beans. Plant Dis., 71(11): 984-990.
Weller DM, Saettler AW (1980). Evaluation of seedborne *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* as primary inocula in bean blights. Phytopathology 70: 148-152.
Zaumeyer WJ, Thomas HR (1957). A monographic study of bean diseases and methods for their control. Washington, U.S.: Department of Agriculture, Technical Bulletin 868.