Identification of the causal agent of soybean bacterial blight
(Pseudomonas savanstanoi pv. glycinea)

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Abstract

Bacterial blight (Pseudomonas savanstanoi pv. glycinea (Coerper 1919) Gardan et al. 1992) is the most common bacterial disease of soybean, has a worldwide distribution and occurs wherever soybean is grown. In seasons with rainy springs, the bacterial blight occurs frequently in Serbia, often with great severity. Total of 20 strains were collected from several locations and different soybean varieties in Serbia during 2011. Morphological and biochemical characteristics were tested according to standard methods. Cultural characteristics were determined on the basis of appearance, size and shape of colonies developed on MPA, NSA and King B nutritive media. For serological identification, monoclonal antibodies of P. s. pv. glycinea (Express Kit, NEOGEN Europe Ltd., Scotland, UK) were used. Polymerase chain reaction (PCR) was preformed with the specific primers pair which directs the amplification of the 650-bp DNA fragment. The following known cultures from National Collection of Plant Pathogenic Bacteria UK (NCPPB 3318 – P. s. pv. glycinea) used as positive and negative control (NCPPB 881 – X. gardneri). Based on pathogenicity, biochemical, serological and molecular tests we concluded that all investigated strains belong to P. s. pv. glycinea species.

Key words: soybean, bacterial blight, Pseudomonas savanstanoi pv. glycinea, PCR

Introduction

Bacterial blight of soybean, caused by Pseudomonas savanstanoi pv. glycinea, is a common and very widespread disease of soybean both in Serbia and throughout the world. In years with rainy springs, the bacterial blight occurs regularly in the province of Vojvodina (Serbia), often with great severity (Balaž et al., 1995; Vidić and Balaž, 1997).

Material i methods

Isolation of bacterial strains

Leaves showing characteristic bacterial blight symptoms were collected from soybean fields located in the major soybean-producing localities in Vojvodina region. Leaves were placed in paper bags and transported to the laboratory. Isolations of the pathogen was carried out from diseased soybean leaves, using standard procedure of smearing macerated tissues across the nutritive surface (Schaad, 1980; Lelliot and Stead, 1987; Arsenijević, 1997).
Hypersensitivity reaction on tobacco leaves (HR)

Pathogenicity of obtained isolates were tested on tobacco plants (cv. Samsun) according to the method of Klement (1963). Water bacterial suspension of each isolate (10⁶ CFU/ml) was infiltrated into the mesophyll of a fully expanded tobacco leaf. HR was observed after 24 h.

Cultural properties

Cultural and biochemical-physiological characteristics were tested according to known methods. Growth characteristics were determined on the basis of colour appearance, size and shape of colonies developed on nutritive MPA and NAS media (Arsenijević, 1997; Schaad et al., 2001). King’s medium B (King et al., 1954) was used for green fluorescent pigment production. Fluorescence was observed under UV light.

Agglutination test (express agglutination test)

For serological identification was used express agglutination test with commercial antibodies of P. s. pv. glycinea (Express Kit, NEOGEN Europe Ltd., Scotland, UK). Positive reaction was clearly indicated as granular agglutination. In the case of negative reaction the drop of test reagent remains transparent (no agglutination). Obtained results were compared with positive and negative controls of Express Kit.

Molecular identification by PCR

Identification of the bacterium P. s. pv. glycinea by molecular means (PCR) is based on the multiplication of DNA fragments controlling the synthesis of the phytotoxin coronatine (Bereswill et al., 1994). The testing was done using a ready-made PCR mixture (Eppendorf Master Mix-Fermentas) for 100 reactions and a set of primers amplifying diagnostic PCR products 650 base pairs (bp) in size. The primer sequences were as follows:

Primer 1: 5’ - GGC GCT CCC TCG CACTT - 3’
Primer 2: 5’ - GGT ATT GGC GGG GGTGC - 3’

Used as a positive control was the coronatine-producing isolate of P. s. pv. glycinea NCPPB 3318. Also included was the isolate X. gardneri (NCPPB 881) as a negative control. Whole cells were used as the DNA sample with no prior DNA isolation. The program was started after closing the lid of the machine. DNA fragments obtained by the chain reaction were detected on a 2% agarose gel to which 9ml of ethidium bromide had been previously added. 10 ml of the PCR product were applied onto each gel plate. Electrophoretic separation of the components was performed at room temperature over a period of 60 minutes using a constant voltage of 100V and maximum current power of 5V/cm. After that, the gel was viewed on a transilluminator and photographed. PCR tests in which specific amplification products 650 bp in size detected were considered positive, provided the same product was not identified in samples used as a negative control or a blank.
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**Results and discussion**

Our study showed that all isolates studied, originating from diseased soybean plants, belong to *Pseudomonas savanstanoi pv. glycinea*. They were very homogeneous in morphological, physiological and biochemical characteristics and did not differ from the reference strain obtained from national collection of plant pathogenic bacteria NCPPB 3318 (UK).

**Hypersensitivity reaction on tobacco leaves (HR)**

Pathogenicity test on tobacco showed that all the tested isolates caused hypersensitive reaction after 24h. The rapid death of plant cells leading to necrosis of inoculated tissue with no appearance of usual watersoaked spots.

**Cultural properties**

Three to four days after the incubation, large, shiny, white, and bulging colonies of bacteria formed on the nutrient medium containing sucrose (NSA). They produce fluorescence on King B medium and small, white, shiny colonies on the surface of MPA.

**Agglutination test (express agglutination test)**

Formation of granular sediment (agglutination) revealed positive reaction in all tested isolate, so we can conclude that our bacterial isolates belong to *P. s. pv. glycinea* species. Agglutination tests are more sensitive than other precipitin tests and can be carried out with lower concentrations of reactants than are necessary for precipitation tests (Koenig et al. 1979; Walkey et al. 1992; Hughes and Ollennu 1993).

**Molecular identification method using PCR**

In all the samples from our study, a nucleic acid fragment 650bp in size and specific to *P. s. pv. glycinea* strains producing coronatine was detected using polymerase chain reaction as described by Bereswill et al. (1994). Besides *P. s. pv. glycinea*, there are also several other varieties of the pathogen that are capable of producing coronatine (*pv. tomato, pv. artropurea, pv. maculicola* and *pv. morsprunorum*) (Cuppels et al., 1990; Wiebe and Campbell, 1993).

**Conclusion**

On the basis of pathogenic, morphological, biochemical, physiological, serological and molecular characterization, we concluded that the studied isolates belong to *Pseudomonas savanstanoi pv. glycinea*.

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References


